

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification : Not classified		A2	(11) International Publication Number: WO 00/41546
			(43) International Publication Date: 20 July 2000 (20.07.00)
(21) International Application Number: PCT/US00/00902		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 14 January 2000 (14.01.00)			
(30) Priority Data: 60/116,380 14 January 1999 (14.01.99) US Not furnished 10 January 2000 (10.01.00) US			
(71) Applicant (for all designated States except US): AMYLIN PHARMACEUTICALS, INC. [US/US]; Nancy K. Dahl, 9373 Towne Centre Drive, San Diego, CA 92121 (US).			
(72) Inventors; and (73) Inventors/Applicants (for US only): YOUNG, Andrew [US/US]; P. O. Box 60591, Point Loma, CA 92166 (US). L'ITALIEN, James, J. [US/US]; 15752 Caminito Canteras, Del Mar, CA 92014 (US). KOLTERMAN, Orville [US/US]; 15793 Hidden Valley Drive, Poway, CA 92064 (US).		Published <i>Without international search report and to be republished upon receipt of that report.</i>	

(54) Title: NOVEL EXENDIN AGONIST FORMULATIONS AND METHODS OF ADMINISTRATION THEREOF

EXENDIN-3

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
1 5 10 15
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
20 25 30
Ser Gly Ala Pro Pro Pro Ser-NH₂
35

(57) Abstract

Novel exendin and exendin agonist compound formulations and dosages and methods of administration thereof are provided. These compositions and methods are useful in treating diabetes and conditions that would be benefited by lowering plasma glucose or delaying and/or slowing gastric emptying or inhibiting food intake.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

NOVEL EXENDIN AGONIST FORMULATIONS AND
METHODS OF ADMINISTRATION THEREOF

Related Applications

5 This application claims priority from U.S. Provisional
Application 60/116,380, entitled "Novel Exendin Agonist
Formulations And Methods Of Administration Thereof," filed
January 14, 1999, and U.S. Provisional Application 60/[not yet
assigned], entitled "Use of Exendins and Agonists Thereof for
10 Modulation of Triglyceride Levels and Treatment of
Dyslipidemia," filed January 10, 2000, the contents of which are
hereby incorporated by reference in their entireties.

Field of the Invention

The present invention relates to novel exendin and peptide
15 exendin agonist formulations, dosages, and dosage formulations
that are bioactive and are deliverable via injectable and non-
injectable routes, for example, via the respiratory tract, the
mouth, and the gut. These formulations and dosages and methods
of administration are useful in the treatment of diabetes,
20 including Type I and II diabetes, in the treatment of disorders
which would be benefited by agents which lower plasma glucose

levels, and in the treatment of disorders which would be benefited by the administration of agents useful in delaying and/or slowing gastric emptying or reducing food intake.

BACKGROUND

5 The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art to the presently claimed inventions, or relevant, nor that any of the publications specifically or implicitly referenced
10 are prior art.

 The exendins are peptides that are found in the salivary secretions of the Gila monster and the Mexican Beaded Lizard, reptiles that are indigenous to Arizona and Northern Mexico. Exendin-3 [SEQ. ID. NO. 1: His Ser Asp Gly Thr Phe Thr Ser Asp
15 Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂] is present in the salivary secretions of *Heloderma horridum* (Mexican Beaded Lizard), and exendin-4 [SEQ. ID. NO. 2: His Gly
20 Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂] is present in the salivary secretions of

Heloderma suspectum (Gila monster) (Eng, J., et al., J. Biol. Chem., 265:20259-62, 1990; Eng, J., et al., J. Biol. Chem., 267:7402-05, 1992). The amino acid sequence of exendin-3 is shown in Figure 1. The amino acid sequence of exendin-4 is
5 shown in Figure 2. Exendin-4 was first thought to be a (potentially toxic) component of the venom. It now appears that exendin-4 is devoid of toxicity, and that it instead is made in salivary glands in the Gila monster.

The exendins have some sequence similarity to several
10 members of the glucagon-like peptide family, with the highest homology, 53%, being to GLP-1[7-36]NH₂ [SEQ. ID. NO. 3] (Goke, et al., J. Biol. Chem., 268:19650-55, 1993). GLP-1[7-36]NH₂ is also known as proglucagon[78-107], or simply "GLP-1" as used most often herein. GLP-1 has an insulinotropic effect, stimulating
15 insulin secretion from pancreatic beta cells. GLP-1 has also been reported to inhibit glucagon secretion from pancreatic alpha-cells (Ørsov, et al., Diabetes, 42:658-61, 1993; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). The amino acid sequence of GLP-1 is shown in Figure 3. GLP-1 has been
20 reported to inhibit gastric emptying (Willms B, et al., J Clin Endocrinol Metab 81 (1): 327-32, 1996; Wettergren A, et al., Dig

Dis Sci 38 (4): 665-73, 1993), and gastric acid secretion (Schjoldager BT, et al., Dig Dis Sci 34 (5): 703-8, 1989; O'Halloran DJ, et al., J Endocrinol 126 (1): 169-73, 1990; Wettergren A, et al., Dig Dis Sci 38 (4): 665-73, 1993)). GLP-1[7-37], which has an additional glycine residue at its carboxy terminus, also stimulates insulin secretion in humans (Ørso, et al., Diabetes, 42:658-61, 1993). A transmembrane G-protein adenylate-cyclase-coupled receptor said to be responsible at least in part for the insulinotropic effect of GLP-1 has reportedly been cloned from a beta-cell line (Thorens, Proc. Natl. Acad. Sci. USA 89:8641-45, 1992).

GLP-1 has been the focus of significant investigation in recent years due to reported actions such as the amplification of stimulated insulin production (Byrne MM, Goke B. Lessons from human studies with glucagon-like peptide-1: Potential of the gut hormone for clinical use. In: Fehmann HC, Goke B. Insulinotropic Gut Hormone Glucagon-Like Peptide 1. Basel, Switzerland: Karger, 1997:219-33), the inhibition of gastric emptying (Wettergren A, et al., Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man, Dig. Dis. Sci. 1993 Apr;38(4):665-73), the inhibition of

glucagon secretion (Creutzfeldt WOC, et al., Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7-36) amide in type I diabetic patients, Diabetes Care 1996;19(6):580-6), and a purported role in

5 appetite control (Turton MD, et al., A role for glucagon-like peptide-1 in the central regulation of feeding, Nature 1996 Jan;379(6560):69-72). GLP-1 has also been reported to restore islet glucose sensitivity in aging rats, restoring their glucose tolerance to that of younger rats (Egan JM, et al., Glucagon-like peptide-1 restores acute-phase insulin release to aged
10 rats, Diabetologia 1997 Jun;40(Suppl 1):A130). The short duration of biological action of GLP-1 *in vivo* is one feature of the peptide that has hampered its development as a therapeutic agent.

15 Pharmacological studies have demonstrated both similarities and differences between exendin-4 and GLP-1. Exendin-4 reportedly can act at GLP-1 receptors on insulin-secreting β TC1 cells, at dispersed acinar cells from guinea pig pancreas, and at parietal cells from stomach. The peptide is also reported to
20 stimulate somatostatin release and inhibit gastrin release in isolated stomachs (Goke, et al., J. Biol. Chem. 268:19650-55,

1993; Schepp, et al., Eur. J. Pharmacol., 69:183-91, 1994;
Eissele, et al., Life Sci., 55:629-34, 1994). Exendin-3 and
exendin-4 were reportedly found to stimulate cAMP production in,
and amylase release from, pancreatic acinar cells (Malhotra, R.,
5 et al., Regulatory Peptides, 41:149-56, 1992; Raufman, et al.,
J. Biol. Chem. 267:21432-37, 1992; Singh, et al., Regul. Pept.
53:47-59, 1994). Exendin-4 also has a significantly longer
duration of action than GLP-1. For example, in one experiment,
glucose lowering by exendin-4 in diabetic mice was reported to
10 persist for several hours, and, depending on dose, for up to 24
hours (Eng J. Prolonged effect of exendin-4 on hyperglycemia of
db/db mice, Diabetes 1996 May; 45(Suppl 2):152A (abstract 554)).
Based on their insulinotropic activities, the use of exendin-3
and exendin-4 for the treatment of diabetes mellitus and the
15 prevention of hyperglycemia has been proposed (Eng, U.S. Patent
No. 5,424,286).

C-terminally truncated exendin peptides such as exendin-
4[9-39], a carboxyamidated molecule, and fragments 3-39 through
9-39 have been reported to be potent and selective antagonists
20 of GLP-1 (Goke, et al., J. Biol. Chem., 268:19650-55, 1993;
Raufman, J.P., et al., J. Biol. Chem. 266:2897-902, 1991;

Schepp, W., et al., Eur. J. Pharm. 269:183-91, 1994; Montrose-Rafizadeh, et al., Diabetes, 45(Suppl. 2):152A, 1996). Exendin-4[9-39] is said to block endogenous GLP-1 in vivo, resulting in reduced insulin secretion. Wang, et al., J. Clin. Invest., 5 95:417-21, 1995; D'Alessio, et al., J. Clin. Invest., 97:133-38, 1996). A receptor apparently responsible for the insulinotropic effect of GLP-1 in rats has reportedly been cloned from rat pancreatic islet cell (Thorens, B., Proc. Natl. Acad. Sci. USA 89:8641-8645, 1992). Exendins and exendin-4[9-39] are said to 10 bind to the cloned rat GLP-1 receptor (rat pancreatic β -cell GLP-1 receptor (Fehmann HC, et al., Peptides 15 (3): 453-6, 1994) and human GLP-1 receptor (Thorens B, et al., Diabetes 42 (11): 1678-82, 1993)). In cells transfected with the cloned GLP-1 receptor, exendin-4 is reportedly an agonist, i.e., it increases 15 cAMP, while exendin[9-39] is identified as an antagonist, i.e., it blocks the stimulatory actions of exendin-4 and GLP-1. Id.

Exendin-4[9-39] is also reported to act as an antagonist of the full length exendins, inhibiting stimulation of pancreatic acinar cells by exendin-3 and exendin-4 (Raufman, et al., J. Biol. Chem. 266:2897-902, 1991; Raufman, et al., J. Biol. Chem., 20 266:21432-37, 1992). It is also reported that exendin[9-39]

inhibits the stimulation of plasma insulin levels by exendin-4,
and inhibits the somatostatin release-stimulating and gastrin
release-inhibiting activities of exendin-4 and GLP-1 (Kolligs,
F., et al., Diabetes, 44:16-19, 1995; Eissele, et al., Life
5 Sciences, 55:629-34, 1994). Exendin [9-39] has been used to
investigate the physiological relevance of central GLP-1 in
control of food intake (Turton, M.D. et al. Nature 379:69-72,
1996). GLP-1 administered by intracerebroventricular injection
inhibits food intake in rats. This satiety-inducing effect of
10 GLP-1 delivered ICV is reported to be inhibited by ICV injection
of exendin [9-39] (Turton, supra). However, it has been
reported that GLP-1 does not inhibit food intake in mice when
administered by peripheral injection (Turton, M.D., Nature
379:69-72, 1996; Bhavsar, S.P., Soc. Neurosci. Abstr. 21:460
15 (188.8), 1995).

The results of an investigation of whether exendins are the
species homolog of mammalian GLP-1 was reported by Chen and
Drucker who cloned the exendin gene from the Gila monster (J.
Biol. Chem. 272(7):4108-15 (1997)). The observation that the
20 Gila monster also has separate genes for proglucagons (from
which GLP-1 is processed), that are more similar to mammalian

proglucagon than exendin, indicates that exendins are not species homologs of GLP-1.

Agents that serve to delay gastric emptying have found a place in medicine as diagnostic aids in gastrointestinal radiological examinations. For example, glucagon is a polypeptide hormone that is produced by the alpha cells of the pancreatic islets of Langerhans. It is a hyperglycemic agent that mobilizes glucose by activating hepatic glycogenolysis. It can to a lesser extent stimulate the secretion of pancreatic insulin. Glucagon is used in the treatment of insulin-induced hypoglycemia, for example, when administration of glucose intravenously is not possible. However, as glucagon reduces the motility of the gastro-intestinal tract it is also used as a diagnostic aid in gastrointestinal radiological examinations.

Glucagon has also been used in several studies to treat various painful gastrointestinal disorders associated with spasm. Daniel, et al. (Br. Med. J., 3:720, 1974) reported quicker symptomatic relief of acute diverticulitis in patients treated with glucagon compared with those who had been treated with analgesics or antispasmodics. A review by Glauser, et al. (J. Am. Coll. Emergency Physns, 8:228, 1979) described relief of

acute esophageal food obstruction following glucagon therapy.

In another study, glucagon significantly relieved pain and tenderness in 21 patients with biliary tract disease compared with 22 patients treated with placebo (M.J. Stower, et al., Br.

5 J. Surg., 69:591-2, 1982).

Methods for regulating gastrointestinal motility using amylin agonists are described in commonly owned International Application No. PCT/US94/10225, published March 16, 1995.

Methods for regulating gastrointestinal motility using
10 exendin agonists are described in commonly owned U.S. Patent Application Serial No. 08/908,867, filed August 8, 1997 entitled "Methods for Regulating Gastrointestinal Motility," which application is a continuation-in-part of U.S. Patent Application Serial No. 08/694,954 filed August 8, 1996.

15 Methods for reducing food intake using exendin agonists are described in commonly owned U.S. Patent Application Serial No. 09/003,869, filed January 7, 1998, entitled "Use of Exendin and Agonists Thereof for the Reduction of Food Intake," which claims the benefit of U.S. Provisional Application Nos. 60/034,905
20 filed January 7, 1997, 60/055,404 filed August 7, 1997, 60/065,442 filed November 14, 1997 and 60/066,029 filed November

14, 1997.

Exendins have also been reported to have inotropic and diuretic effects, as set forth in commonly owned International Application No. PCT/US99/02554, filed February 5, 1999, claiming
5 the benefit of Provisional Application No. 60/075,122, filed February 13, 1998.

Novel exendin agonist compounds are described in commonly owned PCT Application Serial No. PCT/US98/16387 filed August 6, 1998, entitled "Novel Exendin Agonist Compounds," which claims
10 the benefit of U.S. Patent Application Serial No. 60/055,404, filed August 8, 1997.

Other novel exendin agonists are described in commonly owned PCT Application Serial No. PCT/US98/24210, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds," which
15 claims the benefit of U.S. Provisional Application No. 60/065,442 filed November 14, 1997.

Still other novel exendin agonists are described in commonly owned PCT Application Serial No. PCT/US98/24273, filed November 13, 1998, entitled "Novel Exendin Agonist Compounds,"
20 which claims the benefit of U.S. Provisional Application No. 60/066,029 filed November 14, 1997.

Since the appearance of the first therapeutically active peptides and proteins produced by genetic engineering, there has been an ever-increasing demand to be able to deliver these drugs by routes other than parenteral. This has been thwarted, however, by the very properties of peptides and proteins that set them apart from the small drug molecules widely in use today. These properties include molecular size, susceptibility to proteolytic breakdown, rapid plasma clearance, peculiar dose-response curves, immunogenicity, biocompatibility, and the tendency of peptides and proteins to undergo aggregation, adsorption, and denaturation.

It is generally understood that the administration of peptide drugs by routes other than subcutaneous or intravenous injection, or intravenous infusion, is often not practical because of, for example, in the case of oral administration, both enzymatic degradation and non-absorption in the gastrointestinal tract. Thus, there continues to exist a need for the development of alternative methods to the inconvenient, sometimes painful, injection for administration of peptide drugs, such as exendins and the peptide exendin agonist analogs referenced above. In addition to formulations and dosages

useful in the administration of exendins and exendin agonists by injection, formulations, dosage formulations, and methods that solve these problems and that are useful in the non-injection delivery of therapeutically effective amounts of exendin and
5 exendin agonists are described and claimed herein.

The contents of the above-identified articles, patents, and patent applications, and all other documents mentioned or cited herein, are hereby incorporated by reference in their entirety. Applicants reserve the right to physically incorporate into this
10 application any and all materials and information from any such articles, patents, patent applications, or other documents mentioned or cited herein.

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides
15 novel exendin and exendin agonist compound formulations and dosages thereof exhibiting advantageous properties that include effects in slowing gastric emptying and lowering plasma glucose levels. Thus, this aspect of the invention includes formulations of exendins and exendin agonists that comprise an
20 exendin or exendin agonist mixed together with a buffer (preferably an acetate buffer), an iso-osmolality modifier

(preferably mannitol), and optionally containing a preservative (preferably m-cresol), said formulation having a pH of between about 3.0 and about 7.0 (preferably between about 4.0 and about 5.0). By an "exendin agonist" is meant a compound that mimics
5 one or more effects of exendin, for example, by binding to a receptor where exendin causes one or more of these effects, or by activating a signaling cascade by which exendin causes one or more of these effects. Exendin agonists include exendin agonist peptides, such as analogs and derivatives of exendin-3 and
10 exendin-4 that have one or more desired activities of exendin. Various exendin agonist analogs are identified or referenced herein.

Additional exendin and exendin agonist formulations within the scope of the invention include a parenteral liquid dosage
15 form, a lyophilized unit-dosage form, a lyophilized multi-use dosage form, and modifications of these dosage forms that are useful in the oral, nasal, buccal, sublingual, intra-tracheal, and pulmonary delivery of exendins and exendin agonists.

Thus, the invention includes parenteral liquid dosage forms
20 that comprise approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or from about

0.005 to about 0.05% (w/v), respectively of the active ingredient in an aqueous system along with approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate or similar buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0, as well as either approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol iso-osmolality modifier (preferably mannitol) or up to about 0.9% saline or a combination of both leading to an isotonic or an iso-osmolar solution in an aqueous continuous phase. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol is also present if the formulation is packaged in a multi-use container. A sufficient amount of water for injection is added to obtain the desired concentration of solution. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, must maintain the overall stability of the active ingredient. Useful polyhydric alcohols include such compounds as sorbitol, mannitol, glycerol, and polyethylene glycols

(PEGs). The polyhydric alcohols and the carbohydrates will also be effective in stabilizing protein against denaturation caused by elevated temperature and by freeze-thaw or freeze-drying processes. Suitable carbohydrates include galactose, arabinose, 5 lactose or any other carbohydrate which does not have an adverse affect on a diabetic patient, if intended for that use, i.e., the carbohydrate is not metabolized to form large concentrations of glucose in the blood. Preferably, the peptides of the present invention are admixed with a polyhydric alcohol such as 10 sorbitol, mannitol, inositol, glycerol, xylitol, and polypropylene/ethylene glycol copolymer, as well as various polyethylene glycols (PEG) of molecular weight 200, 400, 1450, 3350, 4000, 6000, and 8000). Mannitol is the preferred polyhydric alcohol.

15 The lyophilized unit-dose formulations of the present invention are also stable, but need not be isotonic and/or iso-osmolar. They include active ingredient(s), a bulking agent to facilitate cake formation (which may also act as a tonicifer and/or iso-osmolality modifier upon reconstitution to either 20 facilitate stability of the active ingredient and/or lessen the pain on injection), and may also include a surfactant that

benefits the properties of the cake and/or facilitates reconstitution. The lyophilized unit-dose formulations of the present invention include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or 0.005 to 5 0.05% (w/v) of the active ingredient. It may not be necessary to include a buffer in the formulation and/or to reconstitute the lyophile with a buffer if the intention is to consume the contents of the container within the stability period established for the reconstituted active ingredient. If a 10 buffer is used, it may be included in the lyophile or in the reconstitution solvent. Therefore, the formulation and/or the reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer either alone or in combination to 15 obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol iso-osmolality modifier (as described above) or up to 20 0.9% saline or a combination of both leading to a isotonic or iso-osmolar solution in the reconstituted aqueous phase. A

surfactant, preferably about 0.1 to about 1.0% (w/v) of polysorbate 80 or other non-ionic detergent, may be included. As noted above, sodium chloride, as well as other excipients, may also be present in the lyophilized unit-dosage formulation, 5 if desired. The liquid formulation of the invention prior to lyophilization will be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation of isotonic and/or iso-osmolar solutions after reconstitution.

The invention also includes lyophilized and liquid multi- 10 dose formulations. As with the parenteral liquid and lyophilized unit-dosage formulations described above, the lyophilized multi-unit-dosage form should contain a bulking agent to facilitate cake formation. A preservative is included to facilitate multiple use by the patient. These dosage forms 15 include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or from about 0.005 to 0.05% (w/v), respectively of the active ingredient. If a buffer is used, it may be included in the lyophile or in the reconstitution solvent, and the formulation and/or the 20 reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate,

citrate or glutamate buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either

5 approximately 1.0 to 10% (w/v) of a carbohydrate or a polyhydric alcohol iso-osmolality modifier (preferably mannitol) or up to 0.9% saline, or a combination of both, leading to an isotonic or iso-osmolar solution in the reconstituted aqueous phase. A surfactant, preferably about 0.1 to about 1.0% (w/v) of

10 polysorbate 80 or other non-ionic detergent, may be included. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol (preferably m-cresol) is also present if the formulation

15 is packaged in a multi-use container. Sodium chloride, as well as other excipients, may also be present, if desired. The liquid formulation of the invention should be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation of isotonic and/or iso-osmolar solutions after

20 reconstitution.

The invention further includes solid dosage forms useful

for oral, buccal, sublingual, intra-tracheal, nasal, and pulmonary delivery. The formulations that best support pulmonary and/or intra-tracheal dosage forms may be either preserved or unpreserved liquid formulations and/or dry powder formulations. The preserved or unpreserved liquid formulations will be essentially identical to the formulations described above under preserved or unpreserved liquid parenteral formulations. The pH of the solution should be about 3.0 to 7.0, more specifically from about 4.0 to 6.0, or from about 4.0 to 5.0, with a pH greater than or equal to about 5.0 being most preferred to reduce the potential for bronchoconstriction. The dry powder formulations may contain a bulking agent and/or salts to facilitate particle size formation and appropriate particle size distribution. A surfactant and/or salts may also benefit the properties of the particle morphology and/or facilitate tissue uptake of the active ingredient. Dry powder dosage forms can range from 1% to 100% (w/w), respectively of the active ingredient. It may not be necessary to include a bulking agent and/or salts to facilitate particle size formation and/or distribution. The bulking agent and/or salts may consist of either approximately 0 to 99% (w/w) of a carbohydrate or

polyhydric alcohol or approximately 0 to 99% salt or a combination of both leading to the preferred particle size and distribution. A surfactant, preferably about 0.1 to about 1.0% (w/w) of polysorbate 80 or other non-ionic detergent, may be included. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, will maintain the overall stability of the active ingredient and facilitate the proper level of hydration.

Also within the scope of the invention is the formulation comprising up to 50 mg/ml of an exendin or an exendin agonist in 30mM acetate buffer (pH about 4.5) and mannitol, with or without a preservative.

Further within the scope of the invention are preferred dosages for exendins and exendin agonists when given by injection, and when given by other routes. Thus, formulations for exendin and exendin agonists having comparable potency are provided for the administration by injection of from about 0.1 to about 0.5 μ g per kilogram, given one to three times per day. Typically, for the patient with diabetes who weighs in the range from about 70 kilograms (average for the type 1 diabetic) to about 90 kilograms (average for the type 2 diabetic), for

example, this will result in the total administration of about 10 to about 120 μg per day in single or divided doses. If administered in divided doses, the doses are preferably administered two or three times per day, and more preferably, 5 two times per day.

In a preferred injection procedure, the exendin or exendin agonist is administered parenterally, more preferably by injection, for example, by peripheral injection. Preferably, about 1 μg -30 μg to about 1 mg of the exendin or exendin agonist 10 is administered per day. More preferably, about 1-30 μg to about 500 μg , or about 1-30 μg to about 50 μg of the exendin or exendin agonist is administered per day. Most preferably, depending upon the weight of the subject and the potency of the compound administered, about 3 μg to about 50 μg of the exendin or exendin 15 agonist is administered per day. Preferred doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.005 $\mu\text{g}/\text{kg}$ per dose to about 0.2 $\mu\text{g}/\text{kg}$ per dose. More preferably, doses based upon patient weight for compounds having approximately the potency of exendin-4 range from 20 about 0.02 $\mu\text{g}/\text{kg}$ per dose to about 0.1 $\mu\text{g}/\text{kg}$ per dose. Most preferably, doses based upon patient weight for compounds having

approximately the potency of exendin-4 range from about 0.05 µg/kg per dose to about 0.1 µg/kg per dose. These doses are administered from 1 to 4 times per day, preferably from 1 to 2 times per day. Doses of exendins or exendin agonists will
5 normally be lower if given by continuous infusion. Doses of exendins or exendin agonists will normally be higher if given by non-injection methods, such as oral, buccal, sublingual, nasal, pulmonary or skin patch delivery.

Oral dosages according to the present invention will
10 include from about 50 to about 100 times the active ingredient, i.e., from about 500 to about 12,000 µg per day in single or divided doses, preferably from about 500 to about 5,000 µg per day. Pulmonary dosages according to the present invention will include from about 10 to about 100 times the active ingredient,
15 i.e., from about 100 to about 12,000 µg per day in single or divided doses, preferably about 500 to 1000 µg per day. Nasal, buccal and sublingual dosages according to the present invention will also include from about 10 to about 100 times the active ingredient, i.e., from about 100 to about 12,000 µg per day in
20 single or divided doses.

Preferred dosages for nasal administration are from about 10-1000 to about 1200-12,000 μg per day, for buccal administration from about 10-1000 to about 1200-12,000 μg per day, and for sublingual administration from about 10-1000 to about 1200-8,000 μg per day. Sublingual dosages are preferably smaller than buccal dosages. Administration dosages for exendin agonists having less than or greater than the potency of exendin-4 are increased or decreased as appropriate from those described above and elsewhere herein.

Also included within the scope of the present invention are methods of administration of said novel exendin agonist compound formulations and dosages by delivery means alternative to subcutaneous injection or intravenous infusion, including, for example, by nasal delivery, pulmonary delivery, oral delivery, intra-tracheal delivery, sublingual delivery, and buccal delivery.

According to another aspect, the present invention provides novel exendin agonist compound formulations and dosages, and methods for the administration thereof, that are useful in treating diabetes (including type 1 and type 2 diabetes), obesity, and other conditions that will benefit from the

administration of a therapy that can slow gastric emptying, lowering plasma glucose levels, and reduce food intake.

The invention also includes methods for treatment of subjects in order to increase insulin sensitivity by
5 administering an exendin or an exendin agonist. The exendin and exendin agonist formulations and dosages described herein may be used to increase the sensitivity of a subject to endogenous or exogenous insulin.

In one preferred aspect, the exendin or exendin agonist used
10 in the methods of the present invention is exendin-3 [SEQ. ID. NO. 1]. In another preferred aspect, said exendin is exendin-4 [SEQ. ID. NO. 2]. Other preferred exendin agonists include exendin-4 (1-30) [SEQ ID NO 6: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys
15 Asn Gly Gly], exendin-4 (1-30) amide [SEQ ID NO 7: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-NH₂], exendin-4 (1-28) amide [SEQ ID NO 40: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys
20 Asn-NH₂], ¹⁴Leu, ²⁵Phe exendin-4 [SEQ ID NO 9: His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu

Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro
Pro Ser-NH₂], ¹⁴Leu, ²⁵Phe exendin-4 (1-28) amide [SEQ ID NO 41: His
Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu
Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂], and
5 ¹⁴Leu, ²²Ala, ²⁵Phe exendin-4 (1-28) amide [SEQ ID NO 8: His Gly Glu
Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val
Arg Leu Ala Ile Glu Phe Leu Lys Asn-NH₂].

Other features and advantages of the invention will be
apparent from the following description of the preferred
10 embodiments thereof, and from the claims.

In accordance with the present invention and as used
herein, the following terms are defined to have the following
meanings, unless explicitly stated otherwise. "Pharmaceutically
acceptable salt" includes salts of the compounds of the present
15 invention derived from the combination of such compounds and an
organic or inorganic acid. In practice the use of the salt form
amounts to use of the base form. The compounds of the present
invention are useful in both free base and salt form, with both
forms being considered as being within the scope of the present
20 invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the amino acid sequence for exendin-3
[SEQ. ID. NO. 1].

Figure 2 depicts the amino acid sequence for exendin-4
[SEQ. ID. NO. 2].

5 Figure 3 depicts the amino acid sequence for GLP-1[7-36]NH₂
(GLP-1) [SEQ. ID. NO. 3].

Figure 4 depicts the plasma levels of exendin-4 in rats
after intra-tracheal administration.

Figure 5a depicts the plasma exendin-4 concentration after
10 intra-tracheal instillation in db/db mice.

Figure 5b depicts the effect of intra-tracheal
administration of exendin-4 on plasma glucose in db/db mice.

Figures 6a and 6b depict the effect of intra-tracheal
administration of exendin-4 on plasma glucose in ob/ob mice.

15 Figure 7a depicts the plasma exendin-4 concentration after
intra-tracheal instillation into rats.

Figure 7b depicts the bioavailability of exendin-4
following intra-tracheal instillation into rats.

Figure 8 depicts plasma exendin-4 concentrations in rats
20 exposed to aerosolized exendin-4.

Figure 9a depicts the effect of ten minutes of exposure to

aerosolized exendin-4 on plasma glucose in db/db mice.

Figure 9b depicts the plasma exendin-4 concentration after ten minutes of exposure of db/db mice to aerosolized exendin-4.

Figure 10 depicts plasma exendin-4 concentrations in rats
5 after intra-nasal administration of exendin-4.

Figure 11 depicts the effect of intra-gastric administration of exendin-4 on plasma glucose in db/db mice.

Figure 12a depicts the plasma exendin-4 concentration after sublingual administration to db/db mice.

10 Figure 12b depicts the effect of sublingual administration of exendin-4 on plasma glucose in db/db mice.

Figure 12c depicts the plasma exendin-4 concentration after sublingual administration to rats.

15 Figure 12d depicts the bioavailability of exendin-4 after sublingual administration.

Figure 12e depicts the Cmax of sublingual exendin-4.

Figure 13 depicts the effect of exendin-4 (administered i.p. twice daily) on food intake (a), change in body weight (b) (initial body weight $441 \pm 39\text{g}$), or change in hemoglobin A_{1c} (c)
20 ($7.68 \pm 0.20\%$ at 0 weeks). Dose-responses (right panels) are for the means over the last 2 of 6 weeks treatment.

Figure 14 depicts the plasma glucose concentration (a), glucose infusion rate required to maintain euglycemia (b) and plasma lactate concentration (c) in clamp procedures performed on ZDF rats previously treated for 6 weeks with the specified
5 doses of exendin-4 (i.p. twice daily). Dose-responses for glucose infusion rate and plasma lactate concentration are based upon mean values obtained between 60 and 180 min of the clamp procedure.

Figure 15 depicts the amino acid sequences for certain
10 exendin agonist compounds useful in the present invention [SEQ ID NOS 9-39].

Figures 16 and 17 depict glucose-lowering results from the clinical study described in Example 12.

DETAILED DESCRIPTION OF THE INVENTION

15 Exendins and Exendin Agonists

Exendin-3 and Exendin-4 are naturally occurring peptides isolated from the salivary secretions of the Gila monster and the Mexican Beaded Lizard. Animal testing of exendin-4 has shown that its ability to lower blood glucose persists for
20 several hours. Exendin-4, a 39-amino acid polypeptide, is synthesized using solid phase synthesis as described herein, and

this synthetic material has been shown to be identical to that of native exendin-4.

Various aspects of the nonclinical pharmacology of exendin-4 have been studied. In the brain, exendin-4 binds principally to the area postrema and nucleus tractus solitarius region in the hindbrain and to the subfornical organ in the forebrain. Exendin-4 binding has been observed in the rat and mouse brain and kidney. The structures to which exendin-4 binds in the kidney are unknown.

10 A number of other experiments have compared the biologic actions of exendin-4 and GLP-1 and demonstrated a more favorable spectrum of properties for exendin-4. A single subcutaneous dose of exendin-4 lowered plasma glucose in *db/db* (diabetic) and *ob/ob* (diabetic obese) mice by up to 40%. In Diabetic Fatty Zucker (ZDF) rats, 5 weeks of treatment with exendin-4 lowered HbA_{1c} (a measure of glycosylated hemoglobin used to evaluate plasma glucose levels) by up to 41%. Insulin sensitivity was also improved by 76% following 5 weeks of treatment in obese ZDF rats. In glucose intolerant primates, dose-dependent decreases in plasma glucose were also observed. See also Example 6, which describes the results of an experiment indicating that exendin

15
20

is more potent and/or effective than GLP-1 in amplifying glucose-stimulated insulin release. Example 8, furthermore, describes work showing that the ability of exendin-4 to lower glucose in vivo was 3430 times more potent than that of GLP-1.

5 An insulintropic action of exendin-4 has also been observed in rodents, improving insulin response to glucose by over 100% in non-fasted Harlan Sprague Dawley (HSD) rats, and by up to ~10-fold in non-fasted *db/db* mice. Higher pretreatment plasma glucose concentrations were associated with greater
10 glucose-lowering effects. Thus the observed glucose lowering effect of exendin-4 appears to be glucose-dependent, and minimal if animals are already euglycemic. Exendin-4 treatment is also associated with improvement in glycemic indices and in insulin sensitivity, as described in Examples 9 and 13.

15 Exendin-4 dose dependently slowed gastric emptying in HSD rats and was ~90-fold more potent than GLP-1 for this action. Exendin-4 has also been shown to reduce food intake in NIH/Sw (Swiss) mice following peripheral administration, and was at least 1000 times more potent than GLP-1 for this action.
20 Exendin-4 reduced plasma glucagon concentrations by approximately 40% in anesthetized ZDF rats during

hyperinsulinemic, hyperglycemic clamp conditions, but did not affect plasma glucagon concentrations during euglycemic conditions in normal rats. See Example 4. Exendin-4 has been shown to dose-dependently reduce body weight in obese ZDF rats, while in lean ZDF rats, the observed decrease in body weight appears to be transient.

Through effects on augmenting and restoring insulin secretion, as well as inhibiting glucagon secretion, exendin-4 will be useful in people with type 2 diabetes who retain the ability to secrete insulin. Its effects on food intake, gastric emptying, other mechanisms that modulate nutrient absorption, and glucagon secretion also support the utility of exendin-4 in the treatment of, for example, obesity, type 1 diabetes, and people with type 2 diabetes who have reduced insulin secretion.

The toxicology of exendin-4 has been investigated in single-dose studies in mice, rats, and monkeys, repeated-dose (up to 28 consecutive daily doses) studies in rats and monkeys and *in vitro* tests for mutagenicity and chromosomal alterations. To date, no deaths have occurred, and there have been no observed treatment-related changes in hematology, clinical chemistry, or gross or microscopic tissue changes. Exendin-4

was demonstrated to be non-mutagenic, and did not cause chromosomal aberrations at the concentrations tested (up to 5000 $\mu\text{g/mL}$).

In support of the investigation of the nonclinical
5 pharmacokinetics and metabolism of exendin-4, a number of immunoassays have been developed. A radioimmunoassay with limited sensitivity (~ 100 pM) was used in initial pharmacokinetic studies. A two-site IRMA assay for exendin-4 was subsequently validated with a lower limit of quantitation of
10 15 pM. See Examples 5 and 7. The bioavailability of exendin-4, given subcutaneously, was found to be approximately 50-80% using the radioimmunoassay. This was similar to that seen following intraperitoneal administration (48-60%). Peak plasma concentrations (C_{max}) occurred between 30 and 43 minutes (T_{max}).
15 Both C_{max} and AUC values were monotonically related to dose. The apparent terminal half-life for exendin-4 given subcutaneously was approximately 90-110 minutes. This was significantly longer than the 14-41 minutes seen following intravenous dosing. Similar results were obtained using the IRMA assay. Degradation
20 studies with exendin-4 compared to GLP-1 indicate that exendin-4 is relatively resistant to degradation.

Investigation of the structure activity relationship (SAR) to evaluate structures that may relate to the antidiabetic activity of exendin, for its stability to metabolism, and for improvement of its physical characteristics, especially as it pertains to peptide stability and to amenability to alternative delivery systems, has led to the discovery of exendin agonist peptide compounds. Exendin agonists include exendin peptide analogs in which one or more naturally occurring amino acids are eliminated or replaced with another amino acid(s). Preferred exendin agonists are agonist analogs of exendin-4. Particularly preferred exendin agonists those described in International Application No. PCT/US98/16387, filed August 6, 1998, entitled, "Novel Exendin Agonist Compounds," which claims the benefit of United States Provisional Application No. 60/055,404, filed August 8, 1997, including compounds of the formula (I) [SEQ ID NO. 3]:

Xaa₁ Xaa₂ Xaa₃ Gly Thr Xaa₄ Xaa₅ Xaa₆ Xaa₇ Xaa₈
Ser Lys Gln Xaa₉ Glu Glu Glu Ala Val Arg Leu
Xaa₁₀ Xaa₁₁ Xaa₁₂ Xaa₁₃ Leu Lys Asn Gly Gly Xaa₁₄
Ser Ser Gly Ala Xaa₁₅ Xaa₁₆ Xaa₁₇ Xaa₁₈-Z

wherein Xaa₁ is His, Arg or Tyr; Xaa₂ is Ser, Gly, Ala or Thr;
Xaa₃ is Asp or Glu; Xaa₄ is Phe, Tyr or naphthylalanine; Xaa₅ is

Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is Asp or Glu; Xaa₈ is Leu, Ile, Val, pentylglycine or Met; Xaa₉ is Leu, Ile, pentylglycine, Val or Met; Xaa₁₀ is Phe, Tyr or naphthylalanine; Xaa₁₁ is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp, Phe, Tyr, or naphthylalanine; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently Pro, homoproline, 3Hyp, 4Hyp, thioproline, N-alkylglycine, N-alkylpentylglycine or N-alkylalanine; Xaa₁₈ is Ser, Thr or Tyr; and Z is -OH or -NH₂; with the proviso that the compound is not exendin-3 or exendin-4.

Preferred N-alkyl groups for N-alkylglycine, N-alkylpentylglycine and N-alkylalanine include lower alkyl groups preferably of 1 to about 6 carbon atoms, more preferably of 1 to 4 carbon atoms. Suitable compounds include those listed in Figure 1 having amino acid sequences of SEQ. ID. NOS. 9 to 39.

Preferred exendin agonist compounds include those wherein Xaa₁ is His or Tyr. More preferably, Xaa₁ is His.

Preferred are those compounds wherein Xaa₂ is Gly.

Preferred are those compounds wherein Xaa₉ is Leu, pentylglycine, or Met.

Preferred compounds include those wherein Xaa₁₃ is Trp or Phe.

Also preferred are compounds where Xaa₄ is Phe or naphthylalanine; Xaa₁₁ is Ile or Val and Xaa₁₄, Xaa₁₅, Xaa₁₆ and

Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-alkylalanine. Preferably N-alkylalanine has a N-alkyl group of 1 to about 6 carbon atoms.

According to an especially preferred aspect, Xaa₁₅, Xaa₁₆ and
5 Xaa₁₇ are the same amino acid residue.

Preferred are compounds wherein Xaa₁₈ is Ser or Tyr, more preferably Ser.

Preferably Z is -NH₂.

According to one aspect, preferred are compounds of formula
10 (I) wherein Xaa₁ is His or Tyr, more preferably His; Xaa₂ is Gly; Xaa₄ is Phe or naphthylalanine; Xaa₉ is Leu, pentylglycine or Met; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile or Val; Xaa₁₄, Xaa₁₅, Xaa₁₆ and Xaa₁₇ are independently selected from Pro, homoproline, thioproline or N-alkylalanine; and Xaa₁₈ is Ser or
15 Tyr, more preferably Ser. More preferably Z is -NH₂.

According to an especially preferred aspect, especially preferred compounds include those of formula (I) wherein: Xaa₁ is His or Arg; Xaa₂ is Gly; Xaa₃ is Asp or Glu; Xaa₄ is Phe or naphthylalanine; Xaa₅ is Thr or Ser; Xaa₆ is Ser or Thr; Xaa₇ is
20 Asp or Glu; Xaa₈ is Leu or pentylglycine; Xaa₉ is Leu or pentylglycine; Xaa₁₀ is Phe or naphthylalanine; Xaa₁₁ is Ile, Val

or t-butyltylglycine; Xaa₁₂ is Glu or Asp; Xaa₁₃ is Trp or Phe;

Xaa₁₄, Xaa₁₅, Xaa₁₆, and Xaa₁₇ are independently Pro,
homoproline, thioproline, or N-methylalanine; Xaa₁₈ is Ser or
Tyr; and Z is -OH or -NH₂; with the proviso that the compound
5 does not have the formula of either SEQ. ID. NOS. 1 or 2. More
preferably, Z is -NH₂. Especially preferred compounds include
those having the amino acid sequence of SEQ. ID. NOS. 9, 10, 21,
22, 23, 26, 28, 34, 35 and 39.

According to an especially preferred aspect, provided are
10 compounds where Xaa₉ is Leu, Ile, Val or pentylglycine, more
preferably Leu or pentylglycine, and Xaa₁₃ is Phe, Tyr or
naphthylalanine, more preferably Phe or naphthylalanine. These
compounds will exhibit advantageous duration of action and be
less subject to oxidative degradation, both in vitro and in
15 vivo, as well as during synthesis of the compound.

Exendin agonist compounds also include those described in
International Application No. PCT/US98/24210, filed November 13,
1998, entitled, "Novel Exendin Agonist compounds," which claims
the benefit of United States Provisional Application No.
20 60/065,442, filed November 14, 1997, including compounds of the
formula (II) [SEQ ID NO. 4]:

Xaa₁ Xaa₂ Xaa₃ Gly Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀

Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀

Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁; wherein

5

Xaa₁ is His, Arg or Tyr;

Xaa₂ is Ser, Gly, Ala or Thr;

Xaa₃ is Asp or Glu;

Xaa₅ is Ala or Thr;

10 Xaa₆ is Ala, Phe, Tyr or naphthylalanine;

Xaa₇ is Thr or Ser;

Xaa₈ is Ala, Ser or Thr;

Xaa₉ is Asp or Glu;

Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;

15 Xaa₁₁ is Ala or Ser;

Xaa₁₂ is Ala or Lys;

Xaa₁₃ is Ala or Gln;

Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;

Xaa₁₅ is Ala or Glu;

20 Xaa₁₆ is Ala or Glu;

Xaa₁₇ is Ala or Glu;

- Xaa₁₉ is Ala or Val;
Xaa₂₀ is Ala or Arg;
Xaa₂₁ is Ala or Leu;
Xaa₂₂ is Ala, Phe, Tyr or naphthylalanine;
5 Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine
or Met;
Xaa₂₄ is Ala, Glu or Asp;
Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;
Xaa₂₆ is Ala or Leu;
10 Xaa₂₇ is Ala or Lys;
Xaa₂₈ is Ala or Asn;
Z₁ is -OH,
-NH₂
Gly-Z₂,
15 Gly Gly-Z₂,
Gly Gly Xaa₃₁-Z₂,
Gly Gly Xaa₃₁ Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,
20 Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,
Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂ or

Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂;

Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently Pro,

homoproline, 3Hyp, 4Hyp, thioproline,

5 N-alkylglycine, N-alkylpentylglycine or

N-alkylalanine; and

Z₂ is -OH or -NH₂;

provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈, Xaa₁₀,

Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁,

10 Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala.

Preferred N-alkyl groups for N-alkylglycine, N-alkylpentylglycine and N-alkylalanine include lower alkyl groups preferably of 1 to about 6 carbon atoms, more preferably of 1 to 4 carbon atoms.

15 Preferred exendin agonist compounds include those wherein

Xaa₁ is His or Tyr. More preferably Xaa₁ is His.

Preferred are those compounds wherein Xaa₂ is Gly.

Preferred are those compounds wherein Xaa₁₄ is Leu, pentylglycine or Met.

20 Preferred compounds are those wherein Xaa₂₅ is Trp or Phe.

Preferred compounds are those where Xaa₆ is Phe or

naphthylalanine; Xaa₂₂ is Phe or naphthylalanine and
Xaa₂₃ is Ile or Val.

Preferred are compounds wherein Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈
are independently selected from Pro, homoproline, thioproline
5 and N-alkylalanine.

Preferably Z₁ is -NH₂.

Preferable Z₂ is -NH₂.

According to one aspect, preferred are compounds of formula
(II) wherein Xaa₁ is His or Tyr, more preferably His; Xaa₂ is
10 Gly; Xaa₆ is Phe or naphthylalanine; Xaa₁₄ is Leu, pentylglycine
or Met; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile or Val;
Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently selected from Pro,
homoproline, thioproline or N-alkylalanine. More preferably Z₁
is -NH₂.

15 According to an especially preferred aspect, especially
preferred compounds include those of formula (II) wherein: Xaa₁
is His or Arg; Xaa₂ is Gly or Ala; Xaa₃ is Asp or Glu; Xaa₅ is
Ala or Thr; Xaa₆ is Ala, Phe or naphthylalanine; Xaa₇ is Thr or
Ser; Xaa₈ is Ala, Ser or Thr; Xaa₉ is Asp or Glu; Xaa₁₀ is Ala,
20 Leu or pentylglycine; Xaa₁₁ is Ala or Ser; Xaa₁₂ is Ala or Lys;
Xaa₁₃ is Ala or Gln; Xaa₁₄ is Ala, Leu or pentylglycine; Xaa₁₅ is

Ala or Glu; Xaa₁₆ is Ala or Glu; Xaa₁₇ is Ala or Glu; Xaa₁₉ is Ala or Val; Xaa₂₀ is Ala or Arg; Xaa₂₁ is Ala or Leu; Xaa₂₂ is Phe or naphthylalanine; Xaa₂₃ is Ile, Val or tert-butylglycine; Xaa₂₄ is Ala, Glu or Asp; Xaa₂₅ is Ala, Trp or Phe; Xaa₂₆ is Ala or Leu; Xaa₂₇ is Ala or Lys; Xaa₂₈ is Ala or Asn; Z₁ is -OH, -NH₂, Gly-Z₂, Gly Gly-Z₂, Gly Gly Xaa₃₁-Z₂, Gly Gly Xaa₃₁ Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂, Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂; Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ being independently Pro homoproline, thioproline or N-methylalanine; and Z₂ being -OH or -NH₂; provided that no more than three of Xaa₃, Xaa₅, Xaa₆, Xaa₈, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇, Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala. Especially preferred compounds include those having the amino acid sequence of SEQ. ID. NOS. 40-61.

According to an especially preferred aspect, provided are compounds where Xaa₁₄ is Leu, Ile, Val or pentylglycine, more preferably Leu or pentylglycine, and Xaa₂₅ is Phe, Tyr or naphthylalanine, more preferably Phe or naphthylalanine. These compounds will be less susceptible to oxidative degradation, both

in vitro and in vivo, as well as during synthesis of the compound.

Exendin agonist compounds also include those described in International Patent Application No. PCT/US98/24273, filed November 13, 1998, entitled, "Novel Exendin Agonist Compounds," which claims the benefit of United States Provisional Application No. 60/066,029, filed November 14, 1997, including compounds of the formula (III) [SEQ ID NO. 5]:

10 Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Xaa₆ Xaa₇ Xaa₈ Xaa₉ Xaa₁₀
Xaa₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Xaa₁₅ Xaa₁₆ Xaa₁₇ Ala Xaa₁₉ Xaa₂₀
Xaa₂₁ Xaa₂₂ Xaa₂₃ Xaa₂₄ Xaa₂₅ Xaa₂₆ Xaa₂₇ Xaa₂₈-Z₁; wherein

Xaa₁ is His, Arg, Tyr, Ala, Norval, Val

15 or Norleu;

Xaa₂ is Ser, Gly, Ala or Thr;

Xaa₃ is Ala, Asp or Glu;

Xaa₄ is Ala, Norval, Val, Norleu or Gly;

Xaa₅ is Ala or Thr;

20 Xaa₆ is Phe, Tyr or naphthylalanine;

Xaa₇ is Thr or Ser;

- Xaa₈ is Ala, Ser or Thr;
- Xaa₉ is Ala, Norval, Val, Norleu, Asp or Glu;
- Xaa₁₀ is Ala, Leu, Ile, Val, pentylglycine or Met;
- Xaa₁₁ is Ala or Ser;
- 5 Xaa₁₂ is Ala or Lys;
- Xaa₁₃ is Ala or Gln;
- Xaa₁₄ is Ala, Leu, Ile, pentylglycine, Val or Met;
- Xaa₁₅ is Ala or Glu;
- Xaa₁₆ is Ala or Glu;
- 10 Xaa₁₇ is Ala or Glu;
- Xaa₁₉ is Ala or Val;
- Xaa₂₀ is Ala or Arg;
- Xaa₂₁ is Ala or Leu;
- Xaa₂₂ is Phe, Tyr or naphthylalanine;
- 15 Xaa₂₃ is Ile, Val, Leu, pentylglycine, tert-butylglycine or Met;
- Xaa₂₄ is Ala, Glu or Asp;
- Xaa₂₅ is Ala, Trp, Phe, Tyr or naphthylalanine;
- Xaa₂₆ is Ala or Leu;
- Xaa₂₇ is Ala or Lys;
- 20 Xaa₂₈ is Ala or Asn;
- Z₁ is -OH,

- NH₂,
 Gly-Z₂,
 Gly Gly-Z₂,
 Gly Gly Xaa₃₁-Z₂,
 5 Gly Gly Xaa₃₁ Ser-Z₂,
 Gly Gly Xaa₃₁ Ser Ser-Z₂,
 Gly Gly Xaa₃₁ Ser Ser Gly-Z₂,
 Gly Gly Xaa₃₁ Ser Ser Gly Ala-Z₂,
 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆-Z₂,
 10 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇-Z₂,
 Gly Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈-Z₂ or Gly
 Gly Xaa₃₁ Ser Ser Gly Ala Xaa₃₆ Xaa₃₇ Xaa₃₈ Xaa₃₉-Z₂; wherein
 Xaa₃₁, Xaa₃₆, Xaa₃₇ and Xaa₃₈ are independently
 Pro, homoproline, 3Hyp, 4Hyp, thioproline,
 15 N-alkylglycine, N-alkylpentylglycine or
 N-alkylalanine; and
 Z₂ is -OH or -NH₂;
 provided that no more than three of Xaa₃, Xaa₄, Xaa₅, Xaa₆,
 Xaa₈, Xaa₉, Xaa₁₀, Xaa₁₁, Xaa₁₂, Xaa₁₃, Xaa₁₄, Xaa₁₅, Xaa₁₆, Xaa₁₇,
 20 Xaa₁₉, Xaa₂₀, Xaa₂₁, Xaa₂₄, Xaa₂₅, Xaa₂₆, Xaa₂₇ and Xaa₂₈ are Ala; and
 provided also that, if Xaa₁ is His, Arg or Tyr, then at least one

of Xaa₃, Xaa₄ and Xaa₅ is Ala.

Preparation of Compounds

The compounds that constitute active ingredients of the formulations and dosages of the present invention may be prepared using standard solid-phase peptide synthesis techniques and preferably an automated or semiautomated peptide synthesizer. The preparation of exendin-3 and exendin-4 is described in Examples 1 and 2 below. The preparation of additional exendin agonist peptide analogs is described in Examples 13-198 below.

Typically, using automated or semiautomated peptide synthesis techniques, an α -N-carbamoyl protected amino acid and an amino acid attached to the growing peptide chain on a resin are coupled at room temperature in an inert solvent such as dimethylformamide, N-methylpyrrolidinone or methylene chloride in the presence of coupling agents such as dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in the presence of a base such as diisopropylethylamine. The α -N-carbamoyl protecting group is removed from the resulting peptide-resin using a reagent such as trifluoroacetic acid or piperidine, and the coupling reaction repeated with the next

desired N-protected amino acid to be added to the peptide chain. Suitable N-protecting groups are well known in the art, with t-butyloxycarbonyl (tBoc) and fluorenylmethoxycarbonyl (Fmoc) being preferred herein.

- 5 The solvents, amino acid derivatives and 4-methylbenzhydryl-amine resin used in the peptide synthesizer may be purchased from Applied Biosystems Inc. (Foster City, CA). The following side-chain protected amino acids may be purchased from Applied Biosystems, Inc.: Boc-Arg(Mts), Fmoc-Arg(Pmc), Boc-
- 10 Thr(Bzl), Fmoc-Thr(t-Bu), Boc-Ser(Bzl), Fmoc-Ser(t-Bu), Boc-Tyr(BrZ), Fmoc-Tyr(t-Bu), Boc-Lys(Cl-Z), Fmoc-Lys(Boc), Boc-Glu(Bzl), Fmoc-Glu(t-Bu), Fmoc-His(Trt), Fmoc-Asn(Trt), and Fmoc-Gln(Trt). Boc-His(BOM) may be purchased from Applied Biosystems, Inc. or Bachem Inc. (Torrance, CA). Anisole,
- 15 dimethylsulfide, phenol, ethanedithiol, and thioanisole may be obtained from Aldrich Chemical Company (Milwaukee, WI). Air Products and Chemicals (Allentown, PA) supplies HF. Ethyl ether, acetic acid, and methanol may be purchased from Fisher Scientific (Pittsburgh, PA).
- 20 Solid phase peptide synthesis may be carried out with an automatic peptide synthesizer (Model 430A, Applied Biosystems

Inc., Foster City, CA) using the NMP/HOBt (Option 1) system and tBoc or Fmoc chemistry (see, Applied Biosystems User's Manual for the ABI 430A Peptide Synthesizer, Version 1.3B July 1, 1988, section 6, pp. 49-70, Applied Biosystems, Inc., Foster City, CA) with capping. Boc-peptide-resins may be cleaved with HF (-5 °C to 0°C, 1 hour). The peptide may be extracted from the resin with alternating water and acetic acid, and the filtrates lyophilized. The Fmoc-peptide resins may be cleaved according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc., 1990, pp. 6-12). Peptides may also be assembled using an Advanced Chem Tech Synthesizer (Model MPS 350, Louisville, Kentucky).

Peptides may be purified by RP-HPLC (preparative and analytical) using a Waters Delta Prep 3000 system. A C4, C8 or C18 preparative column (10 μ , 2.2 x 25 cm; Vydac, Hesperia, CA) may be used to isolate peptides, and purity may be determined using a C4, C8 or C18 analytical column (5 μ , 0.46 x 25 cm; Vydac). Solvents (A=0.1% TFA/water and B=0.1% TFA/CH₃CN) may be delivered to the analytical column at a flow rate of 1.0 ml/min and to the preparative column at 15 ml/min. Amino acid analyses may be performed on the Waters Pico Tag system and processed

using the Maxima program. Peptides may be hydrolyzed by vapor-phase acid hydrolysis (115°C, 20-24 h). Hydrolysates may be derivatized and analyzed by standard methods (Cohen, et al., The Pico Tag Method: A Manual of Advanced Techniques for Amino Acid
5 Analysis, pp. 11-52, Millipore Corporation, Milford, MA (1989)). Fast atom bombardment analysis may be carried out by M-Scan, Incorporated (West Chester, PA). Mass calibration may be performed using cesium iodide or cesium iodide/glycerol. Plasma desorption ionization analysis using time of flight detection
10 may be carried out on an Applied Biosystems Bio-Ion 20 mass spectrometer. Electrospray mass spectroscopy may be carried and on a VG-Trio machine.

Peptide active ingredient compounds useful in the formulations and dosages of the invention may also be prepared
15 using recombinant DNA techniques, using methods now known in the art. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor (1989).

Utility

The formulations and dosages described herein are useful in
20 view of their pharmacological properties. In particular, the formulations and dosages of the invention are effective as

exendins and exendin agonists, and possess activity as agents to lower blood glucose, and to regulate gastric motility and to slow gastric emptying, as evidenced by the ability to reduce post-prandial glucose levels in mammals.

5 Formulation and Administration

Exendin and exendin agonist formulations and dosages of the invention are useful in view of their exendin-like effects, and may conveniently be provided in the form of formulations suitable for parenteral (including intravenous, intramuscular
10 and subcutaneous) administration. Also described herein are formulations and dosages useful in alternative delivery routes, including oral, nasal, buccal, sublingual and pulmonary.

Compounds useful in the invention can be provided as parenteral compositions for injection or infusion. Generally,
15 they can, for example, be suspended in an inert oil, suitably a vegetable oil such as sesame, peanut, olive oil, or other acceptable carrier. Preferably, they are suspended in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about 3.0 to about 7.0, more specifically from about 4.0
20 to 6.0, and preferably from about 4.0 to about 5.0. These compositions may be sterilized by conventional sterilization

techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH buffering agents. Useful buffers include for example, sodium acetate/acetic acid buffers. The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

The exendin and exendin agonist compounds can also be formulated as pharmaceutically acceptable salts (e.g., acid addition salts) and/or complexes thereof. Pharmaceutically acceptable salts are non-toxic salts at the concentration at which they are administered. The preparation of such salts can facilitate the pharmacological use by altering the physical-chemical characteristics of the composition without preventing the composition from exerting its physiological effect.

Examples of useful alterations in physical properties include lowering the melting point to facilitate transmucosal administration and increasing the solubility to facilitate the

administration of higher concentrations of the drug.

Pharmaceutically acceptable salts include acid addition salts such as those containing sulfate, hydrochloride, phosphate, sulfamate, acetate, citrate, lactate, tartrate, 5 methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, cyclohexylsulfamate and quinate.

Pharmaceutically acceptable salts can be obtained from acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric 10 acid, malonic acid, methanesulfonic acid, ethane sulfonic acid, benzene sulfonic acid, p-toluenesulfonic acid, cyclohexyl sulfamic acid, and quinic acid. Such salts may be prepared by, for example, reacting the free acid or base forms of the product with one or more equivalents of the appropriate base or acid in 15 a solvent or medium in which the salt is insoluble, or in a solvent such as water which is then removed in vacuo or by freeze-drying or by exchanging the ions of an existing salt for another ion on a suitable ion exchange resin.

Generally, carriers or excipients can also be used to 20 facilitate administration of the dosages of the present invention. Examples of carriers and excipients include calcium

carbonate, calcium phosphate, various sugars such as lactose, or types of starch, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and physiologically compatible solvents.

If desired, solutions of the above dosage compositions may
5 be thickened with a thickening agent such as methylcellulose. They may be prepared in emulsified form, either water in oil or oil in water. Any of a wide variety of pharmaceutically acceptable emulsifying agents may be employed including, for example, acacia powder, a non-ionic surfactant (such as a
10 Tween), or an ionic surfactant (such as alkali polyether alcohol sulfates or sulfonates, e.g., a Triton).

In general, formulations and dosage compositions of the invention are prepared by mixing the ingredients following generally accepted procedures. For example, the selected
15 components may be simply mixed in a blender or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration and viscosity by the addition of water or thickening agent and possibly a buffer to control pH or an additional solute to control tonicity.

20 Other pharmaceutically acceptable carriers and their formulation are described in standard formulation treatises,

e.g., Remington's Pharmaceutical Sciences by E.W. Martin. See also Wang, Y.J. and Hanson, M.A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, 5 Supp. 42:2S (1988).

For use by the physician, the compounds will be provided in dosage unit form containing an amount of an exendin agonist, with or without another therapeutic agent, for example, a glucose-lowering agent, a gastric emptying modulating agent, a 10 lipid lowering agent, or a food intake inhibitor agent. Therapeutically effective amounts of an exendin agonist for use in the control of blood glucose or in the control of gastric emptying and in conditions in which gastric emptying is beneficially slowed or regulated are those that decrease post- 15 prandial blood glucose levels, preferably to no more than about 8 or 9 mM or such that blood glucose levels are reduced as desired. In diabetic or glucose intolerant individuals, plasma glucose levels are higher than in normal individuals. In such individuals, beneficial reduction or "smoothing" of post- 20 prandial blood glucose levels may be obtained. As will be recognized by those in the field, an effective amount of

therapeutic agent will vary with many factors including the patient's physical condition, the blood sugar level or level of inhibition of gastric emptying to be obtained, or the desired level of food intake reduction, and other factors.

5 Such pharmaceutical compositions are useful in causing increased insulin sensitivity in a subject and may be used as well in disorders, such as diabetes, where sensitivity to insulin is beneficially increased.

10 A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal injection or other form of delivery.

15 The effective daily doses of the compounds are described. The exact dose to be administered may be determined by the attending clinician and may be further dependent upon the efficacy of the particular exendin or exendin agonist compound used, as well as upon the age, weight and condition of the individual. The optimal mode of administration of compounds of
20 the present application to a patient depend on factors known in the art such as the particular disease or disorder, the desired

effect, and the type of patient. While the compounds will typically be used to treat human patients, they may also be used to treat similar or identical diseases in other vertebrates such as other primates, farm animals such as swine, cattle and
5 poultry, and sports animals and pets such as horses, dogs and cats.

The invention includes formulations of exendins and exendin agonists that comprise an exendin or exendin agonist mixed together with a buffer (preferably an acetate buffer), an iso-
10 osmolality modifier (preferably mannitol), and optionally containing a preservative (preferably m-cresol), said formulation having a pH of between about 3.0 and about 7.0 (preferably between about 4.0 and about 5.0).

The formulation which best supports a parenteral liquid
15 dosage form is one in which the active ingredient(s) is stable with adequate buffering capacity to maintain the pH of the solution over the intended shelf life of the product. The dosage form should be either an isotonic and/or an iso-osmolar solution to either facilitate stability of the active ingredient
20 or lessen the pain on injection or both. Devices that deliver very small injection volumes, however, may not require that the

formulation be either isotonic and/or iso-osmolar. If the dosage form is packaged as a unit-dose, then a preservative may be included but is not required. If, however, the dosage form is packaged in a multi-use container, then a preservative is
5 necessary.

These dosage forms include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or from about 0.005 to about 0.05% (w/v), respectively of the active ingredient in an aqueous system along with approximately 0.02 to
10 0.5% (w/v) of an acetate, phosphate, citrate or glutamate or similar buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0, as well as either approximately 1.0 to 10% (w/v) of a
15 carbohydrate or polyhydric alcohol iso-osmolality modifier (preferably mannitol) or up to about 0.9% saline or a combination of both leading to an isotonic or an iso-osmolar solution in an aqueous continuous phase. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the
20 group consisting of m-cresol, benzyl alcohol, methyl ethyl, propyl and butyl parabens and phenol is also present if the

formulation is packaged in a multi-use container. A sufficient amount of water for injection is added to obtain the desired concentration of solution. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, must maintain the overall stability of the active ingredient.

Polyhydric alcohols and carbohydrates share the same feature in their backbones, i.e., -CHOH-CHOH-. The polyhydric alcohols include such compounds as sorbitol, mannitol, glycerol, and polyethylene glycols (PEGs). These compounds are straight-chain molecules. The carbohydrates, such as mannose, ribose, trehalose, maltose, glycerol, inositol, glucose and lactose, on the other hand, are cyclic molecules that may contain a keto or aldehyde group. These two classes of compounds will also be effective in stabilizing protein against denaturation caused by elevated temperature and by freeze-thaw or freeze-drying processes. Suitable carbohydrates include galactose, arabinose, lactose or any other carbohydrate which does not have an adverse affect on a diabetic patient, i.e., the carbohydrate is not metabolized to form large concentrations of glucose in the blood. Such carbohydrates are well known in the art as suitable

for diabetics.

Preferably, the peptides of the present invention are admixed with a polyhydric alcohol such as sorbitol, mannitol, inositol, glycerol, xylitol, and polypropylene/ethylene glycol copolymer, as well as various polyethylene glycols (PEG) of
5 molecular weight 200, 400, 1450, 3350, 4000, 6000, and 8000). Mannitol is the preferred polyhydric alcohol.

The liquid formulation of the invention should be substantially isotonic and/or iso-osmolar. An isotonic solution
10 may be defined as a solution that has a concentration of electrolytes, or a combination of electrolytes and non-electrolytes that will exert equivalent osmotic pressure as that into which it is being introduced, here for example in the case of parenteral injection of the formulation, a mammalian tissue.
15 Similarly, an iso-osmolar solution may be defined as a solution that has a concentration of non-electrolytes that will exert equivalent osmotic pressure as that into which it is being introduced. As used herein, "substantially isotonic" means within $\pm 20\%$ of isotonicity, preferably within $\pm 10\%$. As used
20 herein, "substantially iso-osmolar" means within $\pm 20\%$ of iso-osmolality, preferably within $\pm 10\%$. The formulated product for

injection is included within a container, typically, for example, a vial, cartridge, prefilled syringe or disposable pen.

The formulation which best support a unit-dose parenteral lyophilized dosage form is one in which the active ingredient is reasonably stable, with or without adequate buffering capacity to maintain the pH of the solution over the intended shelf life of the reconstituted product. The dosage form should contain a bulking agent to facilitate cake formation. The bulking agent may also act as a tonicifer and/or iso-osmolality modifier upon reconstitution to either facilitate stability of the active ingredient and/or lessen the pain on injection. As noted above, devices that deliver very small injection volumes may not require the formulation to be isotonic and/or iso-osmolar. A surfactant may also benefit the properties of the cake and/or facilitate reconstitution.

These dosage forms include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or 0.005 to 0.05% (w/v) of the active ingredient. It may not be necessary to include a buffer in the formulation and/or to reconstitute the lyophile with a buffer if the intention is to consume the contents of the container within the stability

period established for the reconstituted active ingredient. If a buffer is used, it may be included in the lyophile or in the reconstitution solvent. Therefore, the formulation and/or the reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either approximately 1.0 to 10% (w/v) of a carbohydrate or polyhydric alcohol iso-osmolality modifier (as described above) or up to 0.9% saline or a combination of both leading to a isotonic or iso-osmolar solution in the reconstituted aqueous phase. A surfactant, preferably about 0.1 to about 1.0% (w/v) of polysorbate 80 or other non-ionic detergent, may be included. As noted above, sodium chloride, as well as other excipients, may also be present in the lyophilized unit-dosage formulation, if desired. Such excipients, however, must maintain the overall stability of the active ingredient. The formulation will be lyophilized within the validation parameters identified to maintain stability of the active ingredient.

The liquid formulation of the invention before lyophilization should be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation of isotonic and/or iso-osmolar solutions after reconstitution. The formulation should be used within the period established by shelf-life studies on both the lyophilized form and following reconstitution. The lyophilized product is included within a container, typically, for example, a vial. If other containers are used such as a cartridge, pre-filled syringe, or disposable pen, the reconstitution solvent may also be included.

As with the parenteral liquid and lyophilized unit-dosage formulations described above, the formulation which best supports a multi-dose parenteral lyophilized dosage form is one in which the active ingredient is reasonably stable with adequate buffering capacity to maintain the pH of the solution over the intended "in-use" shelf-life of the product. The dosage form should contain a bulking agent to facilitate cake formation. The bulking agent may also act as a tonicifer and/or iso-osmolality modifier upon reconstitution to either facilitate stability of the active ingredient or lessen the pain on injection or both. Again, devices that deliver very small

injection volumes may not require the formulation to be either isotonic and/or iso-osmolar. A preservative is, however, necessary to facilitate multiple use by the patient.

These dosage forms include approximately 0.005 to about 0.4%, more specifically from about 0.005 to about 0.02%, or from about 0.005 to 0.05% (w/v), respectively of the active ingredient. It may not be necessary to include a buffer in the formulation and/or to reconstitute the lyophile with a buffer if the intention is to consume the contents of the container within the stability period established for the reconstituted active ingredient. If a buffer is used, it may be included in the lyophile or in the reconstitution solvent. Therefore, the formulation and/or the reconstitution solvent may contain individually or collectively approximately 0.02 to 0.5% (w/v) of an acetate, phosphate, citrate or glutamate buffer either alone or in combination to obtain a pH of the final composition of approximately 3.0 to 7.0, more specifically from about pH 4.0 to about 6.0, or from about 4.0 to 5.0. The bulking agent may consist of either approximately 1.0 to 10% (w/v) of a carbohydrate or a polyhydric alcohol iso-osmolality modifier (preferably mannitol) or up to 0.9% saline, or a combination of

both, leading to an isotonic or iso-osmolar solution in the reconstituted aqueous phase. A surfactant, preferably about 0.1 to about 1.0% (w/v) of polysorbate 80 or other non-ionic detergent, may be included. Approximately 0.005 to 1.0% (w/v) of an anti-microbial preservative selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl and butyl parabens and phenol (preferably m-cresol) is also present if the formulation is packaged in a multi-use container. Sodium chloride, as well as other excipients, may also be present, if desired. Again, however, such excipients must maintain the overall stability of the active ingredient. The formulation should be lyophilized within the validation parameters identified to maintain stability of the active ingredient. The liquid formulation of the invention should be substantially isotonic and/or iso-osmolar either before lyophilization or to enable formation of isotonic and/or iso-osmolar solutions after reconstitution. The formulation should be used within the period established by shelf-life studies on both the lyophilized form and following reconstitution. The lyophilized product is included within a container, typically, for example, a vial. If other containers are used such as a

cartridge, pre-filled syringe or disposable pen, the reconstitution solvent may also be included.

The formulations that best support oral, nasal, pulmonary and/or intra-tracheal dosage forms may be either preserved or unpreserved liquid formulations and/or dry powder or, for oral administration, solid formulations. The preserved or unpreserved liquid formulations will be essentially identical to the formulations described above under preserved or unpreserved liquid parenteral formulations. The pH of the solution should be about 3.0 to 7.0, with a pH greater than or equal to about 5.0 being most preferred to reduce the potential for bronchoconstriction. The dry powder formulations may contain a bulking agent and/or salts to facilitate particle size formation and appropriate particle size distribution. A surfactant and/or salts may also benefit the properties of the particle morphology and/or facilitate tissue uptake of the active ingredient.

These dry powder dosage forms can range from 1% to 100% (w/w), respectively of the active ingredient. It may not be necessary to include a bulking agent and/or salts to facilitate particle size formation and/or distribution. The bulking agent and/or salts may consist of either approximately 0 to 99% (w/w)

of a carbohydrate or polyhydric alcohol or approximately 0 to 99% salt or a combination of both leading to the preferred particle size and distribution. A surfactant, preferably about 0.1 to about 1.0% (w/w) of polysorbate 80 or other non-ionic detergent, may be included. Sodium chloride, as well as other excipients, may also be present, if desired. Such excipients, however, must maintain the overall stability of the active ingredient and facilitate the proper level of hydration.

The formulations that best support nasal and/or intra-tracheal dosage forms may be either preserved or unpreserved liquid dosage formulations described previously.

Dissolvable gels and/or patches may be used to facilitate buccal delivery. The gels may be prepared from various types of starch and/or cellulose derivatives.

Sublingual delivery may be best supported by liquid formulations similar to those described above as parenteral liquid or parenteral lyophilized formulations after reconstitution except without the need for the dosage form to be an isotonic and/or iso-osmolar solution. Solid dosage forms may be similar to oral solid dosage forms except that they must be readily dissolvable under the tongue.

Oral delivery may be best supported by a liquid (gel cap) formulation that is similar to the parenteral liquid formulation except that the solution may be more concentrated and may contain additional additives to facilitate uptake of the active ingredient by the small intestine. Solid dosage forms will contain inert ingredients along with the active ingredient to facilitate tablet formation. These ingredients may include polyhedral alcohols (such as mannitol), carbohydrates, or types of starch, cellulose derivatives, and/or other inert, physiologically compatible materials. The tablet may be enterically coated to minimize digestion in the stomach and thereby facilitate dissolution and uptake further along the alimentary canal.

The invention also includes preferred dosages for exendins and exendin agonists when given by injection, and when given by other routes. Thus, formulations for exendin and exendin agonists having comparable potency are prepared for the administration by injection and include from about 0.1 to about 0.5 μg per kilogram, given one to three times per day. Typically, for the patient with diabetes who weighs in the range from about 70 kilograms (average for the type 1 diabetic) to

about 90 kilograms (average for the type 2 diabetic), for example, this will result in the total administration of about 10 to about 120 μg per day in single or divided doses. If administered in divided doses, the doses are preferably administered two or three times per day, and more preferably, two times per day.

In a preferred injection procedure, the exendin or exendin agonist is administered parenterally, more preferably by injection, for example, by peripheral injection. Preferably, about 1 μg -30 μg to about 1 mg of the exendin or exendin agonist is administered per day. More preferably, about 1-30 μg to about 500 μg , or about 1-30 μg to about 50 μg of the exendin or exendin agonist is administered per day. Most preferably, depending upon the weight of the subject and the potency of the compound administered, about 3 μg to about 50 μg of the exendin or exendin agonist is administered per day. Preferred doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.005 $\mu\text{g}/\text{kg}$ per dose to about 0.2 $\mu\text{g}/\text{kg}$ per dose. More preferably, doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.02 $\mu\text{g}/\text{kg}$ per dose to about 0.1 $\mu\text{g}/\text{kg}$ per dose. Most

preferably, doses based upon patient weight for compounds having approximately the potency of exendin-4 range from about 0.05 µg/kg per dose to about 0.1 µg/kg per dose. These doses are administered from 1 to 4 times per day, preferably from 1 to 2 times per day. Doses of exendins or exendin agonists will normally be lower if given by continuous infusion. Doses of exendins or exendin agonists will normally be higher if given by non-injection methods, such as oral, buccal, sublingual, nasal, pulmonary or skin patch delivery.

10 Oral dosages according to the present invention will include from about 50 to about 100 times the active ingredient, i.e., from about 500 to about 12,000 µg per day in single or divided doses, preferably from about 500 to about 5,000 µg per day. Pulmonary dosages according to the present invention will

15 include from about 10 to about 100 times the active ingredient, i.e., from about 100 to about 12,000 µg per day in single or divided doses, preferably about 500 to 1000 µg per day. Nasal, buccal and sublingual dosages according to the present invention will also include from about 10 to about 100 times the active

20 ingredient, i.e., from about 100 to about 12,000 µg per day in

single or divided doses.

Preferred dosages for nasal administration are from about 10-1000 to about 1200-12,000 μg per day, for buccal administration from about 10-1000 to about 1200-12,000 μg per day, and for sublingual administration from about 10-1000 to about 1200-8,000 μg per day. Sublingual dosages are preferably smaller than buccal dosages. Administration dosages for exendin agonists having less than or greater than the potency of exendin-4 are increased or decreased as appropriate from those described above and elsewhere herein.

Clinical Studies

As described in Example 10 below, a double blind, placebo-controlled single ascending dose study examining the safety, tolerability, and pharmacokinetics of subcutaneous exendin-4 in healthy volunteers has been completed. Five single subcutaneous doses of exendin-4 (0.01, 0.05, 0.1, 0.2 or 0.3 $\mu\text{g}/\text{kg}$) were studied in 40 healthy male volunteers in the fasting state. Maximum plasma exendin-4 concentrations were achieved between one and two hours post-dose with little difference among the doses examined. Examination of the data indicated a dose dependent increase for C_{max} . There were no serious adverse

events reported in this study.

In the healthy male volunteers that participated in this study, exendin-4 was well tolerated at subcutaneous doses up to and including 0.1 $\mu\text{g/kg}$. A decrease in plasma glucose

5 concentration was also observed at this dose. At doses of 0.2 $\mu\text{g/kg}$ and higher, the most commonly observed adverse events were headache, nausea, vomiting, dizziness, and postural hypotension. There was a transient fall in plasma glucose concentration following administration of doses of 0.05 $\mu\text{g/kg}$ and above.

10 Example 12 below describes a further study of the dose-response relationship for the glucose-lowering effect of exendin-4 at doses less than 0.1 $\mu\text{g/kg}$. Fourteen subjects [mean ($\pm\text{SE}$) age 55 ± 2 ; mean BMI ($30.2 \pm 1.6 \text{ kg/m}^2$)] with type 2 diabetes treated with diet \pm oral hypoglycemic agents were
15 studied following withdrawal of oral agents for 10-14 days. Assessments were made following randomized, subcutaneous injection of placebo, 0.01, 0.02, 0.05 and 0.1 $\mu\text{g/kg}$ exendin-4 on separate days following an overnight fast. Injections were given immediately before ingestion of a standardized Sustacal®
20 meal (7kcal/kg) followed by collection of plasma glucose samples at frequent intervals during the subsequent 300 minutes.

The glycemic response was quantified as the time-weighted mean (\pm SE) change in plasma glucose concentration during the 5-hr period. The response ranged from a $+42.0 \pm 7.9$ mg/dL increment above the fasting glucose concentration for placebo compared to
5 a 30.5 ± 8.6 mg/dL decrement below the fasting glucose concentration with $0.1 \mu\text{g/kg}$ exendin-4.

The ED_{50} for this glucose lowering effect was $0.038 \mu\text{g/kg}$. Exendin-4 doses less than $0.1 \mu\text{g/kg}$ appeared to disassociate the glucose lowering effects from the gastrointestinal side effects.
10 Example 12 shows that exendin-4 was not only well tolerated at doses less than $0.1 \mu\text{g/kg}$, but that these doses substantially lowered postprandial plasma glucose concentrations (ED_{50} of $0.038 \mu\text{g/kg}$) in people with type 2 diabetes.

Alternate Routes of Delivery

15 The feasibility of alternate routes of delivery for exendin-4 has been explored by measuring exendin-4 in the circulation in conjunction with observation of a biologic response, such as plasma glucose lowering in diabetic animals, after administration. Passage of exendin-4 has been
20 investigated across several surfaces, the respiratory tract (nasal, tracheal, and pulmonary routes) and the gut (sublingual,

gavage and intraduodenal routes). Biologic effect and appearance of exendin-4 in blood have been observed with each route of administration via the respiratory tract, and with sublingual and gavaged peptide via the gastrointestinal tract.

- 5 *Intra-tracheal Administration* - As described herein, intra-tracheal administration of exendin-4 into fasted rats (20 µg/50 µL/animal) produced a rise in the mean plasma exendin-4 concentration to 2060±960 pg/mL within 5-10 minutes after administration. Elevated plasma exendin-4 concentrations were
- 10 maintained for at least 1 hour after instillation (see Figure 4). In diabetic *db/db* mice, intra-tracheal instillation of exendin-4 (1 µg/animal) lowered plasma glucose concentration by 30% while that in the vehicle control group increased by 41% 1.5 hours after treatment. In these animals the mean plasma
- 15 concentration of exendin-4 was 777±365 pg/ml at 4.5 hours after treatment (see Figures 5a and 5b).

In diabetic *ob/ob* mice, intra-tracheal instillation of exendin-4 (1 µg/animal) decreased plasma glucose concentration to 43% of the pre-treatment level after 4 hours while that in

20 the vehicle control group was not changed (see Figures 6a and 6b).

Nine overnight-fasted male Sprague Dawley rats (age 96-115 days, weight 365-395, mean 385g) were anesthetized with halothane, tracheotomized, and catheterized via the femoral artery. At t=0 min, 30 μ L of saline in which was dissolved 2.1 μ g (n=3), 21 μ g (n=3) or 210 μ g of exendin-4 was instilled into the trachea beyond the level of intubation. Blood samples were taken after 5, 10, 20, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min, centrifuged and plasma stored at -20°C for subsequent immunoradiometric (IRMA) assay directed to N-terminal and C-terminal epitopes of the intact exendin-4 molecule. Following intra-tracheal administration, 61-74% of peak plasma concentration was observed within 5 min. T_{max} occurred between 20 and 30 min after administration. AUC and C_{max} were proportional to dose. At a dose of 2.1 μ g (1.5 nmol/kg), resulting in plasma concentrations of ~50pM (where glucose-lowering effects in man are observed), bioavailability was 7.3%. The coefficient of variation was 44%. At higher doses, bioavailability was slightly lower, and the CV was higher (see Figures 7a and 7b). Via the tracheal route of administration, the t_{1/2} (defined pragmatically as time for plasma to fall below 50% of C_{max}) was 30-60 min for the lowest dose and 60-90 min for

the 2 higher doses. In sum, biologically effective quantities of exendin-4 are rapidly absorbed via the trachea without evoking apparent respiratory distress. The respiratory tract is a viable route of administration of exendin-4.

5 *Pulmonary Administration* - Increased plasma concentrations of exendin-4 were detected in rats exposed to aerosolized exendin-4. Exposure of rats to approximately 8 ng of aerosolized exendin-4 per mL of atmosphere for 10 minutes resulted in peak plasma exendin-4 concentrations of 300-1900
10 pg/mL 5 minutes following treatment (see Figure 8). Similar exposure of diabetic *db/db* mice to aerosolized exendin-4 lead to a 33 % decrease in plasma glucose concentration after 1 hour, when a mean plasma exendin-4 concentration of 170 ± 67 pg/mL was detected. Diabetic *db/db* mice in the control group exposed to
15 aerosolized saline recorded no change in plasma glucose (see Figures 9a and 9b).

Nasal administration - Application of exendin-4 into the nasal cavity of rats led to a rise in plasma concentrations. Peak values of 300 pg/mL and 6757 pg/mL were detected 10 minutes
20 after administration of 1 μ g and 100 μ g exendin-4 (dissolved in 2 μ L saline), respectively (see Figure 10).

Administration via the Gut- Male db/db mice (approximately 50g body wt.) were fasted for 2h and before and after an intra-gastric administration of saline or exendin-4 (exendin-4). A 9% decrease in plasma glucose concentration was observed with 1mg/200 μ l/animal and a 15% decrease was observed with 3 mg/200 μ l/animal, compared with a 10% increase plasma glucose in the controls one hour after treatment (see Figure 11).

Sublingual Administration - Sublingual application of exendin-4 (100 μ g/5 μ L/animal) to diabetic db/db mice led to a 15% decrease in plasma glucose concentration one hour after treatment. A 30% increase was observed for the control group receiving saline. The mean exendin-4 plasma level at 60 minutes was 4520 \pm 1846 pg/mL (see Figures 12a, 12b, and 12c).

Eight Sprague Dawley rats (~300g) were briefly anesthetized with metophane while a solution containing 10 μ g/3 μ L (n=4) or 100 μ g/3 μ L (n=4) was pipetted under the tongue. Blood samples were subsequently collected from the topically anesthetized tail and assayed for exendin-4 by IRMA. Plasma concentrations had begun to rise by 3 min after administration and were maximal 10 min and 30 min after administration (10 μ g and 100 μ g doses, respectively). Plasma exendin-4 concentration subsequently

remained above the lower limit of quantitation (LLOQ) beyond 5 hours. Area-under-the-curve to the end of each experiment was calculated by the trapezoidal method. Two numbers were derived, one derived from total immunoreactivity, the other derived from the increment above the non-zero value present at $t=0$. These values were compared to historical intravenous bolus data in the same animal model to obtain, respectively, high and low estimates of bioavailability. For the $10\mu\text{g}$ dose, sublingual bioavailability was 3.1-9.6%, and for a $100\mu\text{g}$ dose, bioavailability was lower at 1.3-1.5%. Variability of AUC was greatest in the first hour after administration (CV 74% and 128% for 10 and $100\mu\text{g}$ doses). For the 5-hour integral, coefficient of variation of the AUC was 20% and 64%, respectively. Peak plasma concentration (C_{max}) occurred as rapidly after sublingual administration as after subcutaneous administration (T_{max} ~30 min). C_{max} after sublingual administration of $10\mu\text{g}$ exendin-4 was 1.5% that after an intravenous bolus, but 14.5% of that obtained after a subcutaneous bolus. C_{max} after sublingual administration of $100\mu\text{g}$ exendin-4 was only 0.29% of that observed after an intravenous bolus, and 6.1% of that obtained after a subcutaneous bolus (see Figures 12d and 12e). Thus,

exendin-4 can be delivered at bioeffective doses via the sublingual route. Bioavailability and C_{max} were greatest, T_{max} was shortest, and variability of availability was least with the lowest sublingual dose. The lowest sublingual dose resulted in
5 plasma concentrations similar to those that are predicted to be effective in lowering glucose in humans (~50-100 pM).

To assist in understanding the present invention the following Examples are included which describe the results of a series of experiments. The experiments relating to this
10 invention should not, of course, be construed as specifically limiting the invention and such variations of the invention, now known or later developed, which would be within the purview of one skilled in the art are considered to fall within the scope of the invention as described herein and hereinafter claimed.

15 EXAMPLE 1 - PREPARATION OF EXENDIN-3

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 1]

The above amidated peptide was assembled on 4-(2'-4'-
20 dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected

amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. Deprotection (Fmoc group removal) of the growing peptide chain was achieved using
5 piperidine. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.)
10 The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 75%.

Used in purification steps and analysis of Examples 1 and 2
15 were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined
20 isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical

RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.2 minutes.

EXAMPLE 2 - PREPARATION OF EXENDIN-4

5 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly
Pro Ser Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 2]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
10 MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Exendin-3 as describe in Example 1. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-
15 HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.9 minutes. Electrospray Mass Spectrometry (M): calculated 4186.6; found 8186.0 to 4186.8 (four lots).

20

EXAMPLE 3 - Exendin-4 IS A CIRCULATING,

MEAL-RELATED PEPTIDE IN THE GILA MONSTER

This experiment investigated whether exendin-4 has a metabolic role in the Gila monster lizard itself. To investigate whether exendin-4 appeared in the blood of the Gila monster in response to feeding, blood was sampled from one
5 animal fasted for 7 weeks, before and 30 min after ingestion of a small rat. Plasma was assayed for full-length exendin-4 using an immunoradiometric assay with monoclonal antibody pairs directed to epitopes at N- and C-termini of exendin-4. Fasting plasma exendin-4 concentration was 76 pg/mL, near the lower
10 limit of quantitation. After eating, this value rose 300-fold to 23,120 pg/mL.

In a second experiment, serial samples were taken from two animals fasted five weeks before and after ingestion of one or two small rats (47-49 g). Plasma exendin-4 concentration rose
15 23- to 36-fold (to 4860, 8340 pg/mL) immediately after eating, consistent with a direct passage of exendin-4 from the salivary gland to blood. After eating a second rat (t=30 min), plasma exendin-4 concentration in one Gila rose further to 27,209 pg/mL. Plasma exendin-4 concentration decayed with a $t_{1/2}$ of 5.00
20 and 5.33 hours, respectively. In conclusion, exendin-4, known to originate from the salivary gland of the Gila monster,

appears in high concentration in the blood immediately after eating. This may represent a meal-related signal to inhibit further eating and promote nutrient storage.

EXAMPLE 4 - EXENDIN-4 DECREASES GLUCAGON SECRETION DURING

5 HYPERGLYCEMIC CLAMPS IN DIABETIC FATTY ZUCKER RATS

Absolute or relative hyperglucagonemia is often a feature of type 1 and type 2 diabetes mellitus, and the suppression of excessive glucagon secretion is a potential benefit of therapy using glucagonostatic agents. In this Example, the effect of
10 exendin-4 on glucagon secretion in male anaesthetized Diabetic Fatty Zucker (ZDF) rats was examined. Using an hyperinsulinemic hyperglycemic clamp protocol, factors tending to influence glucagon secretion were held constant. Plasma glucose was clamped at ~34mM 60 min before beginning intravenous infusions
15 of saline (n=7) or exendin-4 (0.21 μ g + 2.1 μ g/mL/h; n=7). Plasma glucagon concentration measured before these infusions were similar in both groups (306 \pm 30pM versus 252 \pm 32pM, respectively; n.s.).

Mean plasma glucagon concentration in exendin-4 infused
20 rats was nearly half of that in saline-infused rats in the final 60 minutes of the clamp (165 \pm 18pM versus 298 \pm 26pM,

respectively; $P < 0.002$). The hyperglycemic clamp protocol also enabled measurement of insulin sensitivity. Glucose infusion rate during the clamp was increased by $111 \pm 7\%$ in exendin-4-treated versus control rats ($P < 0.001$). In other words, exendin-4 exhibited a glucagonostatic effect in ZDF rats during hyperglycemic clamp studies, an effect that will be of therapeutic benefit in diabetic humans.

EXAMPLE 5 - PHARMACOKINETICS OF EXENDIN-4

IN THE RAT FOLLOWING INTRAVENOUS,

10 SUBCUTANEOUS AND INTRAPERITONEAL ADMINISTRATION

This Example describes work to define the plasma pharmacokinetics of exendin-4 in rats (~350g body weight each) following 2.1, 21, 210 $\mu\text{g}/\text{rat}$ i.v. bolus, s.c. and i.p. administration and 2.1, 21, 210 $\mu\text{g}/\text{hr}/\text{rat}$ i.v. infusion (3 hr).
15 Serial samples of plasma (~120 μL) were assayed using a validated immunoradiometric assay (IRMA). This sandwich-type assay uses mouse-based monoclonal antibodies that react with exendin-4 but do not react with GLP-1 or tested metabolites of exendin-4 or GLP-1. The lower limit of quantitation was 15pM (63pg/mL). The
20 estimated $t_{1/2}$ for exendin-4 was 18-41 min for i.v. bolus, 28-49 for i.v. continuous, 90-216 min for s.c. and 125-174 min for

i.p. injection. Bioavailability was 65-76% for s.c. and i.p. injection. Clearance determined from the i.v. infusion was 4-8 mL/min. Both C_{max} and AUC values within each route of administration were proportional to dose. Volume of distribution was 457-867 mL. Clearance and bioavailability were not dose dependent. C_{max} (or steady-state plasma concentration; C_{ss}) is shown in the table below

Cmax or C _{ss} (nM)				
Route	Intravenous bolus	Intravenous infusion	Subcutaneous	Intraperitoneal
Dose				
2.1 µg	2.9 ± 0.4	1.1 ± 0.1	0.56 ± 0.12	0.26 ± 0.04
21 µg	70 ± 3	19 ± 1.9	4.1 ± 1.5	3.9 ± 1
210 µg	645 ± 12	262 ± 60	28 ± 4	35 ± 6

EXAMPLE 6 - COMPARISON OF THE INSULINOTROPIC ACTIONS

10 OF EXENDIN-4 AND GLUCAGON-LIKE PEPTIDE-1 (GLP-1) DURING AN INTRAVENOUS GLUCOSE CHALLENGE IN RATS

This experiment compares the insulinotropic actions of synthetic exendin-4 and GLP-1 in vivo following an intravenous (i.v.) glucose challenge in rats. Sprague-Dawley rats (~400g)

were anesthetized with halothane and cannulated via the femoral artery and saphenous vein. Following a 90-min recovery period, saline or peptide (30 pmol/kg/min each) was administered i.v. (1ml/h for 2 hours; n=4-5 for each group). Thirty min after

5 infusion commenced, D-glucose (5.7mmol/kg, 0.8ml) was injected i.v. In saline-treated, exendin-4-treated and GLP-1-treated rats, plasma glucose concentrations were similar before injection (9.3 ± 0.3 , 9.7 ± 0.3 , 10.3 ± 0.4 mM), increased by similar amounts after glucose injection (21.7, 21.3, 23.7 mM), and

10 resulted in a similar 60-min glucose AUC (987 ± 39 , 907 ± 30 , 1096 ± 68 mM•min, respectively). That is, the glycemic stimulus was similar in each treatment group. Plasma insulin concentration in saline-treated rats increased 3.3-fold with the glucose challenge (230 ± 53 to a peak of 765 ± 188 pM). With exendin-

15 4 infusion, the increase in plasma insulin concentration was 6.8-fold (363 ± 60 to 2486 ± 365 pM). With GLP-1 the increase in plasma insulin concentration was 2.9-fold (391 ± 27 to 1145 ± 169 pM), which was similar to that obtained in saline-treated rats. The 60-min insulin AUC in saline-treated rats was 24 ± 6 nM•min, was

20 increased 2.8-fold in exendin-treated rats (67 ± 8 nM•min; $P < 0.003$ versus saline; $P < 0.02$ versus GLP-1) and by 20% in GLP-1-treated

rats (n.s. versus saline). Amplification of glucose-stimulated insulin release by exendin-4 was also tested at infusion rates of 3 and 300pmol/kg/min and shown to be dose-dependent. Thus, exendin-4 is more potent and/or effective than GLP-1 in
5 amplifying glucose-stimulated insulin release in intact rats.

EXAMPLE 7 - DEVELOPMENT AND VALIDATION OF AN IMMUNORADIOMETRIC
ASSAY (IRMA) FOR THE QUANTITATION

OF EXENDIN-4 IN PLASMA AND ITS APPLICATION TO

PRECLINICAL TOXICITY AND PHASE I CLINICAL EVALUATIONS

10 A sensitive and specific sandwich-type immunoradio-metric (IRMA) assay was developed for quantitation of plasma exendin-4 concentration using synthetic exendin-4 as the immunogen. One mouse-derived monoclonal antibody recognizes a C-terminal epitope on exendin-4 (capture antibody) but does not cross-react
15 with GLP-1. The second antibody (detector antibody labeled with ¹²⁵I) recognizes an N-terminal epitope on exendin-4 and GLP-1, and requires a terminal histidine for binding. Thus, the assay as a whole does not detect GLP-1(7-36)NH₂, GLP-1(7-36)COOH or exendin(3-39). Assay validation in rat, monkey, dog, rabbit and
20 human plasmas showed inter- and intra-assay coefficients of variation <20% and <10%, respectively, accuracy of ±15% with

target low, mid and high controls, and lower and upper limits of quantitation of 62.8 and 2512 pg/mL, respectively. Plasma samples from 28-day subcutaneous toxicity evaluations of exendin-4 in rats and monkeys and a Phase I clinical study in
5 normal subjects were evaluated using the IRMA. The C_{max} values in the animals studies are shown in the table below. Human samples from subcutaneous administration of 0.05, 0.1, 0.2 and 0.3 $\mu\text{g/kg}$ yielded C_{max} values of 90, 224, 370 and 587 pg/mL.

C_{max} (pg/mL)			
Dose ($\mu\text{g/kg}$)	10	100	1000
Rat	7,000	127,000	1,180,000
Monkey	20,000	170,000	1,890,000

10 EXAMPLE 8 - COMPARISON OF GLP-1 RECEPTOR BINDING/ACTIVATING AND
 GLUCOSE-LOWERING EFFECTS OF GLP-1 AND EXENDIN-4

Exendin-4 was synthesized by solid phase peptide synthesis techniques and compared to synthetic GLP-1 in terms of *in vitro* binding to, and activation of, GLP-1 receptors, and *in vivo* in
15 terms of lowering plasma glucose in diabetic *db/db* mice. In a plasma membrane preparation of a rat insulinoma cell line (RINm5f) that expresses the GLP-1 receptor, the peptides were

assayed for their ability to bind and displace radiolabeled GLP-1 and for their ability to stimulate the production of cAMP. The relative order of binding potency was found to be GLP-1 > exendin-4. The relative order of cyclase activation was GLP-1 = exendin-4. Affinities, as shown in the table below, differ over a 4- to 5-fold range. In contrast, in vivo glucose lowering potency differed over a 3430-fold range. Exendin-4 was 3430-fold more potent than GLP-1. The in vivo potency of exendin-4 does not match potency at the GLP-1 receptor, and is likely the culmination of an aggregate of properties.

	Binding IC50 (nM)	Cyclase EC50 (nM)	Glucose-lowering ED50 (μ g)
GLP-1	0.15	0.28	20.6
Exendin-4	0.66	0.30	0.006

EXAMPLE 9 - COMPARISON OF GLYCEMIC INDICES

AND INSULIN SENSITIVITY IN PAIR-FED AND

EXENDIN-4-TREATED DIABETIC FATTY ZUCKER RATS

This Example tests whether the beneficial effects of exendin-4 in ZDF rats were secondary to changes in food intake. It compares effects obtained with exendin-4 to effects observed

in saline-treated matched animals who consumed the same amount of food as was eaten by ZDF rats injected subcutaneously twice daily with 10 μ g exendin-4. Plasma glucose and HbA1c were measured weekly for 6 weeks. One day after the last treatment, animals were anesthetized with halothane and subjected to an hyperinsulinemic (50 mU/kg/min) euglycemic clamp. Changes in HbA1c over 6 weeks differed between treatment groups ($P < 0.001$ ANOVA), increasing in *ad lib* fed (n=5) and pair fed (n=5) rats, but decreasing in exendin-4-treated rats (n=5). Similarly, changes in plasma glucose differed between treatment groups ($P < 0.002$ ANOVA), increasing in *ad lib* fed and pair fed ZDF rats, and decreasing in ZDF rats treated with exendin-4. In the final hour of a 3-hour clamp protocol, glucose infusion rate in exendin-4-treated rats tended to be higher than in pair fed (+105%) and *ad lib* fed (+20%) controls, respectively (10.14 ± 1.43 n=5, 8.46 ± 0.87 n=4, 4.93 ± 2.02 mg/kg/min n=3; n.s. $P = 0.09$ ANOVA). Another index of insulin sensitivity, plasma lactate concentration, differed significantly between treatment groups ($P < 0.02$ ANOVA) and was lowest in exendin-4-treated rats. Thus, exendin-4 treatment is associated with improvement in glycemic indices and in insulin sensitivity that

is partly, but not fully, matched in controls fed the same amount of food, indicating that improvements in metabolic control with exendin-4 in ZDF rats are at least partly due to mechanisms beyond caloric restriction.

5 EXAMPLE 10 - CLINICAL STUDIES AND THE STIMULATION OF
 ENDOGENOUS INSULIN SECRETION BY SUBCUTANEOUS SYNTHETIC
 EXENDIN-4 IN HEALTHY OVERNIGHT FASTED VOLUNTEERS

 In a double blind, placebo-controlled single ascending dose clinical trial to explore safety and tolerability and
10 pharmacokinetics of synthetic exendin-4, exendin-4 formulated for subcutaneous injection was evaluated in healthy male volunteers while assessing effects upon plasma glucose and insulin concentrations. Five single subcutaneous doses of exendin-4 (0.01, 0.05, 0.1, 0.2 or 0.3 µg/kg) were studied in 40
15 healthy male volunteers in the fasting state. Maximum plasma exendin-4 concentrations were achieved between 1 and 2 hours post-dose with little difference among the doses examined. Examination of the data indicated a dose dependent increase for C_{max} . There were no serious adverse events reported in this
20 study and in the healthy male volunteers that participated in this study, exendin-4 was well tolerated at subcutaneous doses

up to and including 0.1 µg/kg. A decrease in plasma glucose concentration was also observed at this dose. At doses of 0.2 µg/kg and higher, the most commonly observed adverse events were headache, nausea, vomiting, dizziness, and postural hypotension.

- 5 There was a transient fall in plasma glucose concentration following administration of doses of 0.05 µg/kg and above.

Forty healthy, lean (mean BMI (\pm SE) 22.7 \pm 1.2) subjects aged 18-40 years were randomly assigned to 5 groups. Within each group of 8 subjects, 6 were assigned to exendin-4 and 2 to
10 placebo (PBO). Exendin-4 (0.01, 0.05, 0.1, 0.2 or 0.3 µg/kg) or placebo was administered following an overnight fast and plasma exendin-4, glucose and insulin concentrations monitored along with safety and tolerability. No safety issues were observed. Doses \leq 0.1 µg/kg were tolerated as well as PBO whereas 0.2 and
15 0.3 µg/kg elicited a dose-dependent increase in nausea and vomiting. Peak plasma exendin-4 concentrations rose dose-dependently and following 0.1µg/kg, exendin-4 immunoreactivity persisted for 360 min. Plasma glucose decreased following all doses, except 0.01 µg/kg, reached a nadir by 30 min and returned
20 back to baseline within 180 min. Subjects receiving 0.3 µg/kg

received a caloric beverage 30 minutes after dosing, precluding comparison of their data. Mean change in plasma glucose (0-180 min): 0.03 ± 0.07 , -0.07 ± 0.08 , -0.38 ± 0.14 , -0.85 ± 0.13 and -0.83 ± 0.23 mmol/L for PBO, 0.01, 0.05, 0.1, and 0.2 $\mu\text{g/kg}$ respectively; $P \leq 0.02$ versus PBO. The lowest plasma glucose recorded was 3.4 mmol/L. Corresponding mean changes in plasma insulin (0-120 min) were 0.43 ± 0.59 , 2.37 ± 0.58 , 2.28 ± 0.66 , 4.91 ± 1.23 , and 14.00 ± 3.34 $\mu\text{U/mL}$; $P \leq 0.01$ versus PBO for the 0.1 and 0.2 $\mu\text{g/kg}$ groups. Thus, in healthy, overnight fasted volunteers, subcutaneous injection of exendin-4 (1) presented no safety issues, (2) was well-tolerated at doses ≤ 0.1 $\mu\text{g/kg}$, (3) led to exendin-4 immunoreactivity in plasma for up to 6 hrs, (4) increased plasma insulin and lowered plasma glucose in a dose-dependent manner without inducing hypoglycemia.

15 EXAMPLE 11 - EFFECTIVENESS OF ALTERNATE

DELIVERY OF EXENDIN-4 IN RODENTS

 This Example tested the delivery of exendin-4 by means alternative to injection, and examined its ability to traverse mucosal surfaces in sufficient quantities to exert biological effect. Changes in concentration of plasma glucose and of

20

intact synthetic exendin-4 (measured by a 2-site immunoradiometric assay) were observed in *db/db* mice administered a saline solution containing differing doses of synthetic exendin-4 via the trachea, via an aerosol mist (pulmonary), via gavage (oral), and under the tongue (sublingual). The same routes of administration, as well as intraduodenally and nasally, were tested in rats, and bioavailability was calculated, for example, for sublingual and intra-tracheal routes. Exendin-4 administered via each of the above routes in mice resulted in significant glucose-lowering activity 1 to 4 hours after administration (*db/db* mice intra-tracheal $P < 0.02$; *ob/ob* mice intra-tracheal $P < 0.0002$; *db/db* mice aerosol $P < 0.0001$; gavage $P < 0.002$; sublingual $P < 0.02$). Dose-dependent increases in plasma exendin-4 concentration were up to 777 \pm 365 pg/mL (*db/db* mice intra-tracheal); 170 \pm 67 pg/mL (*db/db* mice aerosol); 4520 \pm 1846 pg/mL (*db/db* mice sublingual). Similarly, in rats, exendin-4 concentrations were observed up to 68,682 \pm 38,661 pg/mL (intra-tracheal); 1900 pg/mL (pulmonary); 6757 pg/mL (nasal); 3,862 \pm 2,844 pg/mL (sublingual); but no apparent absorption or biological activity when delivered intraduodenally. Bioavailability of exendin-4 in saline was

-7.3% at lower doses when delivered via the trachea, where 61-74% of C_{max} was observed within 5 min. Kinetics thereafter were similar to those observed after subcutaneous administration. Bioavailability of exendin-4 in saline delivered under the tongue was 3.1-9.6% at lower doses. These studies support the delivery of exendin-4 and peptide agonist analogs thereof in biologically effective quantities via convenient non-injectable routes.

EXAMPLE 12 - A SINGLE-BLIND, PLACEBO CONTROLLED STUDY ON
THE METABOLIC EFFECTS OF A RANGE OF DOSES OF SYNTHETIC
EXENDIN-4 GIVEN BY SUBCUTANEOUS INJECTION
TO PEOPLE WITH TYPE 2 DIABETES MELLITUS

This Example describes the results of a two-part, single-blind, placebo controlled study to examine the metabolic effects of a range of doses of synthetic exendin-4 given by the subcutaneous route to subjects with Type II diabetes mellitus. The subjects involved in the study were individuals diagnosed with Type II diabetes and being controlled with diet and/or with oral hypoglycemic agents (OHAs) and with HbA_{1c} concentration $\geq 7.0\%$ but $\leq 12.0\%$ at the screening visit.

The study commenced with a screening visit, after which the

subjects taking OHAs were instructed to stop this medication and return to the clinic approximately 14 days later when the effects of the OHA dissipated. Subjects who participated in Part 1 arrived at the clinic the afternoon prior to the first dose and began the three or four scheduled dosing days. Each dosing event was scheduled to be 24 hours apart.

Following consent and screening, subjects were randomly assigned to receive synthetic extendin-4 or placebo. In the first portion of the study, six subjects were confined to an in-patient clinical research unit for three to four days and assigned to one of 4 treatment sequences, where they were to receive each of the following doses: placebo or synthetic extendin-4 at 0.1 or 0.01, or possibly 0.001 $\mu\text{g/kg}$. Doses were administered subcutaneously following an overnight fast. A standardize liquid meal was given 15 minutes after injection of the study medication. The table below illustrates the dosing schedule for Part 1:

	Day 1	Day 2	Day 3	Day 4*
Subject 1	Placebo	0.1 $\mu\text{g/kg}$	0.01 $\mu\text{g/kg}$	0.001 $\mu\text{g/kg}$
Subject 2	Placebo	0.1 $\mu\text{g/kg}$	0.01 $\mu\text{g/kg}$	0.001 $\mu\text{g/kg}$
Subject 3	0.1 $\mu\text{g/kg}$	Placebo	0.01 $\mu\text{g/kg}$	0.001 $\mu\text{g/kg}$

Subject 4	0.1 µg/kg	Placebo	0.01 µg/kg	0.001 µg/kg
Subject 5	0.1 µg/kg	0.01 µg/kg	Placebo	0.001 µg/kg
Subject 6	0.1 µg/kg	0.01 µg/kg	Placebo	0.001 µg/kg

* Will only be completed if an effect on glucose is observed on Day 3.

In the second part of the study, approximately three days after the completion of Part 1, eight subjects were also confined to an in-patient clinical research unit for four days.

- 5 The subjects were different subjects from those who participated in Part 1. The study procedures and schedule of events during Part 2 were consistent with Part 1. The doses were determined after the effect on glucose in Part 1 was analyzed.

- 10 Because there was no significant effect seen at 0.01 µg/kg during Part 1, subjects were dosed according to the following schedule in Part 2:

	Day 1	Day 2	Day 3	Day 4
Group A	Placebo	0.02 µg/kg	0.05 µg/kg	0.1 µg/kg
Group B	0.02 µg/kg	0.1 µg/kg	Placebo	0.05 µg/kg
Group C	0.05 µg/kg	Placebo	0.1 µg/kg	0.02 µg/kg
Group D	0.1 µg/kg	0.05 µg/kg	0.02 µg/kg	Placebo

Subjects who participated in Part 2 began their dosing following review of the data from Part 1 in the same manner. All subjects returned to the clinic 4 to 6 days after discharge from the in-patient unit for a safety reassessment.

5 The synthetic exendin-4 used for the study was a clear colorless sterile solution for subcutaneous injection, formulated in sodium acetate buffer (pH 4.5) and containing 4.3% mannitol as an iso-osmolality modifier. The strength of synthetic exendin-4 injection was 0.1 mg/mL. One mL of solution
10 was supplied in 3 mL vials with rubber stoppers. Placebo solution was made from the same sterile formulation but without the drug substance, synthetic exendin-4.

 The results of the study are shown in Figures 16 and 17. They indicate the ability of various different doses of exendin-
15 4 (0.02 µg/kg, 0.05 µg/kg, and 0.1 µg/kg) to lower blood glucose in people with Type 2 diabetes.

EXAMPLE 13

 This Example describes an experiment to determine a dose-response for the insulin-sensitizing effects of exendin-4 and
20 agonists thereof in Diabetic Fatty Zucker rats. The exendin-4 used in these studies was obtained from Bachem (Torrance, CA;

Cat H8730, Lot 506189), American Peptides (Sunnyvale, CA; Cat 301577, Lot K1005ITI) and from in-house solid-phase synthesis (lot AR1374-11; peptide content 93.3%). Thirty nine male Diabetic fatty Zucker rats (ZDF)/GmiTM-(fa/fa) (age 116±20 days; weight 441±39 g) were assigned to 5 treatment groups: saline injections only (n=9), exendin-4 injections 0.1, 1, 10 or 100 µg (n=9, 10, 6, 5, respectively). Of these, 35 rats were used in hyperinsulinemic euglycemic clamp studies (n=9, 7, 9, 5, 5, respectively). Blood was sampled from the tip of the topically-anesthetized tail (Hurricane brand of 20% topical benzocaine solution, Beutlich, Waukegan, IL) of conscious overnight-fasted rats before treatment and at weekly intervals for 5 weeks during treatment for analysis of hemoglobin A_{1c} (DCA2000 latex immuno-agglutination inhibition, Bayer Diagnostics, Tarrytown, NY). Body weight was measured daily.

After 6 weeks of treatment, -16 hours after the last exendin-4 (or saline) dose, and after an overnight fast, hyperinsulinemic euglycemic clamps (DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Amer J Physiol* 237:E214-23, 1979) were performed on halothane-anesthetized rats. Rats were

thermoregulated, tracheotomized and catheterized via the saphenous vein for infusion of 20% D-glucose and insulin, and via the femoral artery for blood sampling and blood pressure monitoring (P23XL transducer, Spectramed, Oxnard, CA; universal amplifier, Gould, Valley View, OH; A/D conversion, DataTranslation, Wilmington, DE). Insulin (Humulin-R, Eli Lilly, Indianapolis, IN) was infused at 50 mU/kg/min, beginning at $t=-30$ min and continued until $t=+180$ min. Glucose was infused at a variable rate to maintain euglycemia, determined by glucose sampling and analysis at 5 min intervals (immobilized glucose oxidase method; YSI 2300-Stat Analyzer, Yellow Springs, OH). Mean plasma glucose during clamps was 103.9 mg/dL (mean coefficient of variation was 5.8%). Glucose infusion rate data for analysis were taken from $t=60-180$ min when responses had approached a steady state. Plasma lactate data, obtained from an immobilized lactate oxidase sensor incorporated in the glucose analyzer, were also collected.

 Injections were given intraperitoneally at ~8 a.m. and 4 p.m., Monday through Friday, and at ~10 a.m. on Saturday and Sunday.

 Pairwise statistical analyses were performed using

Student's t-test routines (Instat v3.0, GraphPad Software, San Diego, CA) using $P < 0.05$ as the level of significance. Dose-response analyses used 4-parameter logistic regression and general effects were tested using one-way ANOVA (Prism v3.0, GraphPad Software, San Diego, CA).

The results showed that in Diabetic Fatty Zucker rats treated with different doses of exendin-4 for 6 weeks, there was a dose-dependent reduction in food intake (ED_{50} $0.14 \mu\text{g} \pm 0.15$ log; see Fig 13a), and in body weight (ED_{50} $0.42 \mu\text{g} \pm 0.15$ log; see Fig 13b) of up to 27 ± 2 g, representing a $5.6 \pm 0.5\%$ decrease in body weight relative to saline-injected controls.

In this group of rats, the diabetic course appeared progressive, since hemoglobin A_{1c} initially rose in all groups. Injection of exendin-4 nonetheless appeared to dose-dependently arrest and reverse the rise in hemoglobin A_{1c} (see Fig 13c). The exendin-4 dose-response for effect on hemoglobin A_{1c} measured during the last 2 weeks of treatment was generally significant ($P = 0.05$ ANOVA) and specifically at $1 \mu\text{g}$ and $100 \mu\text{g}$ doses ($P < 0.005$, $P < 0.02$ respectively). A similar pattern was observed in relation to fasting plasma triglycerides in the last 2 weeks of treatment, where plasma concentrations were significantly

reduced at all doses by between 51% and 65% ($P < 0.002$ ANOVA).

Thirty five of the 39 rats entered into the study progressed to an hyperinsulinemic, euglycemic clamp -16 hours after their last treatment. Initial fasting plasma glucose concentrations, higher in saline-treated (489 ± 28 mg/dL) than
5 exendin-treated rats, fell with insulin infusion and were subsequently clamped at similar plasma glucose concentrations (105.6 mg/dL at 60-180 min; mean coefficient of variation 4.6%; see Fig 14a). Glucose infusion rate required to maintain
10 euglycemia was dose-dependently increased by prior treatment with exendin-4 (ED_{50} $1.0 \mu\text{g} \pm 0.41$ log; see Fig 14b). Exendin-4 treatment increased glucose infusion rate by up to 48% relative to saline-treated controls.

Plasma lactate concentration before and during the clamp
15 procedure was dose-dependently reduced by prior treatment with exendin-4 (ED_{50} $4 \mu\text{g} \pm 0.25$ log; see Fig 14c). This effect, representing up to a 42% reduction in mean plasma lactate concentration between 60 and 180 minutes of the clamp, appeared primarily due to a reduction in pre-clamp (basal) lactate
20 concentration; increments in plasma lactate during hyperinsulinemia were similar in all treatment groups. There

were no treatment-related differences in mean arterial pressure measured before or during clamp procedures.

- The approximately 50% increase in insulin sensitivity observed after chronic administration of exendin-4 was both
- 5 important and surprising in view of observations that exendin-4 has no acute effect in insulin-sensitive tissues *in vitro* (i.e. no effect on basal or insulin-stimulated incorporation of radiolabeled glucose into glycogen in isolated soleus muscle, or into lipid in isolated adipocytes; Pittner et al., unpublished).
- 10 Although the possibility that the increase in insulin sensitivity may have resulted in some part from improved glycemic control and reduced glucose toxicity may not be overlooked, it has been reported that the increase in insulin sensitivity from various antidiabetic therapies, including those
- 15 not classed as insulin sensitizing, is quite variable and it has been reported that acute treatment with GLP-1 appears not to immediately alter insulin sensitivity in humans (Orskov L, Holst JJ, Moller J, Orskov C, Moller N, Alberti KG, Schmitz O: GLP-1 does not acutely affect insulin sensitivity in healthy man.
- 20 *Diabetologia* 39:1227-32, 1996; Ahren B, Larsson H, Holst JJ: Effects of glucagon-like peptide-1 on islet function and insulin

sensitivity in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:473-8, 1997; UK Prospective Diabetes Study Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837-53, 1998). Thus chronic administration of exendin-4 appears to be associated with increases in insulin sensitivity that are as great as, if not greater than, those observed with other therapies, including insulin sensitizing drugs such as thiazolidinediones and metformin.

EXAMPLE 14

Preparation of amidated peptide having SEQ. ID. NO. 9

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. However, at some positions coupling was less efficient than expected and double couplings were required. In particular, residues Asp₉, Thr₇, and Phe₆ all required double coupling. Deprotection (Fmoc group

removal) of the growing peptide chain using piperidine was not always efficient. Double deprotection was required at positions Arg₂₀, Val₁₉, and Leu₁₄. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 55%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.5 minutes. Electrospray Mass

Spectrometry (M): calculated 4131.7; found 4129.3.

EXAMPLE 15

Preparation of Peptide having SEO. ID. NO. 10

The above-identified peptide was assembled on 4-(2'-4'-
5 dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,
deprotected and purified in a similar way to Example 14. Used
in analysis were Solvent A (0.1% TFA in water) and Solvent B
10 (0.1% TFA in ACN). Analytical RP-HPLC (gradient 25% to 75%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide gave product peptide having an observed retention time
of 21.5 minutes. Electrospray Mass Spectrometry (M): calculated
4168.6; found 4171.2.

15

EXAMPLE 16

Preparation of Peptide having SEO. ID. NO. 11

The above-identified peptide was assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
20 amino acids (Applied Biosystems, Inc.), cleaved from the resin,
deprotected and purified in a similar way to Example 14. Used

in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 4147.6; found 4150.2.

EXAMPLE 17

Preparation of Peptide having SEQ. ID. NO. 12

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35% to 65% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.7 minutes. Electrospray Mass Spectrometry (M): calculated 4212.6; found 4213.2.

EXAMPLE 18

Preparation of Peptide having SEQ. ID. NO. 13

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 50% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 16.3 minutes. Electrospray Mass Spectrometry (M): calculated 4262.7; found 4262.4.

EXAMPLE 19

Preparation of Peptide having SEQ. ID. NO. 14

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized

peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6

EXAMPLE 20

5 Preparation of Peptide having SEQ. ID. NO. 15

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, 10 deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of 15 the product peptide. Electrospray Mass Spectrometry (M): calculated 4224.7.

EXAMPLE 21

Preparation of Peptide having SEQ. ID. NO. 16

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine 20 MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected

amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6

EXAMPLE 22

10 Preparation of Peptide having SEQ. ID. NO. 17

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4186.6

EXAMPLE 23Preparation of Peptide having SEO. ID. NO. 18

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4200.7.

EXAMPLE 24Preparation of Peptide having SEO. ID. NO. 19

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B

(0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M):
5 calculated 4200.7.

EXAMPLE 25

Preparation of Peptide having SEQ. ID. NO. 20

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
10 MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
15 Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4202.7.

EXAMPLE 26

20

Preparation of Peptide having SEQ. ID. NO. 21

The above-identified peptide is assembled on 4-(2'-4'-

dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4145.6.

EXAMPLE 27

Preparation of Peptide having SEQ. ID. NO. 22

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of

the product peptide. Electrospray Mass Spectrometry (M):
calculated 4184.6.

EXAMPLE 28

Preparation of Peptide having SEQ. ID. NO. 23

5 The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,
deprotected and purified in a similar way to Example 14. Used
10 in analysis are Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide is then carried out to determine the retention time of
the product peptide. Electrospray Mass Spectrometry (M):
15 calculated 4145.6.

EXAMPLE 29

Preparation of Peptide having SEQ. ID. NO. 24

20 The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,

deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4224.7.

EXAMPLE 30

Preparation of Peptide having SEQ. ID. NO. 25

10 The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used
15 in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M):
20 calculated 4172.6.

EXAMPLE 31

Preparation of Peptide having SEQ. ID. NO. 26

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4115.5.

EXAMPLE 32Preparation of Peptide having SEQ. ID. NO. 27

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%

Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4188.6.

5

EXAMPLE 33Preparation of Peptide having SEQ. ID. NO. 28

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4131.6.

10

15

20

EXAMPLE 34Preparation of Peptide having SEQ. ID. NO. 29

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine

MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4172.6.

10

EXAMPLE 35Preparation of Peptide having SEQ. ID. NO. 30

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M):

calculated 4145.6.

EXAMPLE 36

Preparation of Peptide having SEQ. ID. NO. 31

The above-identified peptide is assembled on 4-(2'-4'-
5 dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,
deprotected and purified in a similar way to Example 14.
Additional double couplings are required at the thioproline
10 positions 38, 37, 36 and 31. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical
RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out to
determine the retention time of the product peptide.
15 Electrospray Mass Spectrometry (M): calculated 4266.8.

EXAMPLE 37

Preparation of Peptide having SEQ. ID. NO. 32

The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
20 MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,

deprotected and purified in a similar way to Example 14.

Additional double couplings are required at the thioproline positions 38, 37 and 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-

5 HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 4246.8.

EXAMPLE 38

10 Preparation of Peptide having SEQ. ID. NO. 33

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin,

15 deprotected and purified in a similar way to Example 14.

Additional double couplings are required at the homoproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical

20 RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 4250.8.

EXAMPLE 39

Preparation of Peptide having SEQ. ID. NO. 34

The above-identified peptide is assembled on 4-(2'-4'-
5 dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,
deprotected and purified in a similar way to Example 14.
Additional double couplings are required at the homoproline
10 positions 38, 37, and 36. Used in analysis are Solvent A (0.1%
TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-
HPLC (gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out to
determine the retention time of the product peptide.
15 Electrospray Mass Spectrometry (M): calculated 4234.8.

EXAMPLE 40

Preparation of Peptide having SEQ. ID. NO. 35

The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
20 MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,

deprotected and purified in a similar way to Example 14.

Additional double couplings are required at the thioproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 4209.8.

EXAMPLE 41

10 Preparation of Peptide having SEQ. ID. NO. 36

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, 15 deprotected and purified in a similar way to Example 14. Additional double couplings are required at the homoproline positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 20 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 4193.7.

EXAMPLE 42

Preparation of Peptide having SEO. ID. NO. 37

The above-identified peptide is assembled on 4-(2'-4'-
5 dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,
deprotected and purified in a similar way to Example 14.
Additional double couplings are required at the N-methylalanine
10 positions 38, 37, 36 and 31. Used in analysis are Solvent A
(0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical
RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out to
determine the retention time of the product peptide.
15 Electrospray Mass Spectrometry (M): calculated 3858.2.

EXAMPLE 43

Preparation of Peptide having SEO. ID. NO. 38

The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
20 MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,

deprotected and purified in a similar way to Example 14.

Additional double couplings are required at the N-methylalanine positions 38, 37 and 36. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-

5 HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 3940.3.

EXAMPLE 44

10 Preparation of Peptide having SEQ. ID. NO. 39

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin,

15 deprotected and purified in a similar way to Example 14.

Additional double couplings are required at the N-methylalanine positions 38, 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical

20 RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide.

Electrospray Mass Spectrometry (M): calculated 3801.1.

EXAMPLE 45

Preparation of C-terminal carboxylic acid Peptides corresponding to the above C-terminal amide sequences.

- 5 The above peptides of Examples 1 to 30 are assembled on the so called Wang resin (p-alkoxybenzylalcohol resin (Bachem, 0.54 mmole/g)) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 14. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry provides an experimentally
- 10
- 15 determined (M).

EXAMPLE 46

Preparation of Peptide having SEQ ID NO. 7

- His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
- 20 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-NH₂ [SEQ. ID. NO. 7]

The above amidated peptide was assembled on 4-(2'-4'-

dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. Deprotection (Fmoc group removal) of the growing peptide chain was achieved using piperidine. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 75%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions were pooled

furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 50% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 18.9 minutes. Electrospray Mass Spectrometry (M): calculated 3408.0; found 3408.9.

EXAMPLE 47

Preparation of Peptide having SEQ ID NO. 40

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
10 40]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, 15 deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 40% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time 20 of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 3294.7; found 3294.8.

EXAMPLE 48Preparation of Peptide having SEQ ID NO. 41

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
5 41]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 29% to 36% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed
15 retention time of 20.7 minutes. Electrospray Mass Spectrometry (M): calculated 3237.6; found 3240.

EXAMPLE 49Preparation of Peptide having SEQ ID NO. 42

His Ala Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
20 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 42]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, 5 deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time 10 of 15.2 minutes. Electrospray Mass Spectrometry (M): calculated 3251.6; found 3251.5.

EXAMPLE 50

Preparation of Peptide having SEQ ID NO. 43

His Gly Glu Gly Ala Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu
15 Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID.
NO. 43]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected 20 amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used

in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 13.1 minutes. Electrospray Mass Spectrometry (M): calculated 3207.6; found 3208.3.

EXAMPLE 51

Preparation of Peptide having SEQ ID NO. 44

His Gly Glu Gly Thr Ala Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 44]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
15 amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35% to 45% Solvent B in Solvent A over 30 minutes) of the lyophilized
20 peptide gave product peptide having an observed retention time of 12.8 minutes. Electrospray Mass Spectrometry (M): calculated

3161.5; found 3163.

EXAMPLE 52

Preparation of Peptide having SEQ ID NO. 45

His Gly Glu Gly Thr Phe Thr Ala Asp Leu Ser Lys Gln Leu Glu Glu
5 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
45]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
10 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide gave product peptide having an observed retention time of 15.2 minutes. Electrospray Mass Spectrometry (M): calculated 3221.6; found 3222.7.

EXAMPLE 53

Preparation of Peptide having SEQ ID NO. 46

20 His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.

46]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 34% to 44% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.3 minutes. Electrospray Mass Spectrometry (M): calculated 3195.5; found 3199.4.

EXAMPLE 54

Preparation of Peptide having SEQ ID NO. 47

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 47]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from

the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 15.7 minutes. Electrospray Mass Spectrometry (M): calculated 3221.6; found 3221.6.

EXAMPLE 55

Preparation of Peptide having SEQ ID NO. 48

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 48]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed

retention time of 18.1 minutes. Electrospray Mass Spectrometry (M): calculated 3180.5; found 3180.9.

EXAMPLE 56

5 Preparation of Peptide having SEQ ID NO. 49

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Ala Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
49]

10 The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
15 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.0 minutes. Electrospray Mass Spectrometry
20 (M): calculated 3180.6; found 3182.8.

EXAMPLE 57

Preparation of Peptide having SEQ ID NO. 50

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu

Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 50]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 32% to 42% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.9 minutes. Electrospray Mass Spectrometry (M): calculated 3195.5; found 3195.9.

EXAMPLE 58

15 Preparation of Peptide having SEQ ID NO. 51

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Ala Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 51]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-

protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 17.9 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3179.0.

EXAMPLE 59

Preparation of Peptide having SEQ ID NO. 52

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Ala
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
52]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47% Solvent B in Solvent A over 30 minutes) of the

lyophilized peptide gave product peptide having an observed retention time of 14.3 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3180.0.

EXAMPLE 60

5

Preparation of Peptide having SEQ ID NO. 53

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Ala Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
10 53]

The above-identified peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin,
15 deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time
20 of 13.7 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3179.0.

EXAMPLE 61

Preparation of Peptide having SEQ ID NO. 54

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Ala Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
54]

The above-identified amidated peptide was assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Used in analysis were Solvent A (0.1% TFA in water) and
10 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35%
to 45% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide gave product peptide having an observed
retention time of 14.0 minutes. Electrospray Mass Spectrometry
(M): calculated 3209.6; found 3212.8.

15

EXAMPLE 62Preparation of Peptide having SEQ ID NO. 55

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Ala Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
55]

20

The above-identified amidated peptide was assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and
5 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.3 minutes. Electrospray Mass Spectrometry (M): calculated 3152.5; found 3153.5.

10

EXAMPLE 63Preparation of Peptide having SEQ ID NO. 56

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Ala Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
56]

15

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
20 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35%

to 45% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 12.1 minutes. Electrospray Mass Spectrometry (M): calculated 3195.5; found 3197.7.

5

EXAMPLE 64Preparation of Peptide having SEQ ID NO. 57

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Ala Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
57]

10

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
15 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 10.9 minutes. Electrospray Mass Spectrometry
20 (M): calculated 3179.6; found 3180.5.

EXAMPLE 65

Preparation of Peptide having SEQ ID NO. 58

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn-NH₂ [SEQ. ID. NO.
58]

- 5 The above-identified amidated peptide was assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
10 46. Used in analysis were Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 32%
to 42% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide gave product peptide having an observed
retention time of 17.5 minutes. Electrospray Mass Spectrometry
15 (M): calculated 3161.5; found 3163.0.

EXAMPLE 66Preparation of Peptide having SEQ ID NO. 59

- 20 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Ala Lys Asn-NH₂ [SEQ. ID. NO.
59]

The above-identified amidated peptide was assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 46. Used in analysis were Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 32%
to 42% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide gave product peptide having an observed
retention time of 19.5 minutes. Electrospray Mass Spectrometry
10 (M): calculated 3195.5; found 3199.

EXAMPLE 67

Preparation of Peptide having SEQ ID NO. 60

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Ala Asn-NH₂ [SEQ. ID. NO.
15 60]

The above-identified amidated peptide was assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
20 the resin, deprotected and purified in a similar way to Example
46. Used in analysis were Solvent A (0.1% TFA in water) and

Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 38% to 48% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.5 minutes. Electrospray Mass Spectrometry
5 (M): calculated 3180.5; found 3183.7.

EXAMPLE 68

Preparation of Peptide having SEQ ID-NO. 61

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Ala-NH₂ [SEQ. ID. NO.
10 61]

The above-identified amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
15 the resin, deprotected and purified in a similar way to Example 46. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 34% to 44% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed
20 retention time of 22.8 minutes. Electrospray Mass Spectrometry (M): calculated 3194.6; found 3197.6.

EXAMPLE 69Preparation of Peptide having SEQ ID NO. 62

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
5 Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 62]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
15 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4099.6.

EXAMPLE 70Preparation of Peptide having SEQ ID NO. 63

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
20 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 63]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4042.5.

EXAMPLE 71

Preparation of Peptide having SEQ ID NO. 64

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 64]

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
20 amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used

in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M):
5 calculated 4002.4

EXAMPLE 72

Preparation of Peptide having SEQ ID NO. 65

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Cln Leu Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 65]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
15 the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
20 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

Spectrometry (M): calculated 3945.4.

EXAMPLE 73

Preparation of Peptide having SEQ ID NO. 66

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
5 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 66]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3905.3.

EXAMPLE 74

Preparation of Peptide having SEQ ID NO. 67

20 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser

Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 67]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3848.2.

EXAMPLE 75

Preparation of Peptide having SEQ ID NO. 68

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala-NH₂ [SEQ. ID. NO. 68]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from

the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3808.2.

EXAMPLE 76

Preparation of Peptide having SEQ ID NO. 69

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala-NH₂ [SEQ. ID. NO. 69]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the

retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3751.1.

EXAMPLE 77

Preparation of Peptide having SEQ ID NO. 70

5 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly-NH₂ [SEQ. ID. NO. 70]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
15 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3737.1.

EXAMPLE 78

Preparation of Peptide having SEQ ID NO. 71

20 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu

Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser Gly-NH₂ [SEQ. ID. NO. 71]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
10 to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3680.1.

EXAMPLE 79

15 Preparation of Peptide having SEQ ID NO. 72

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser-NH₂ [SEQ. ID. NO. 72]

The above-identified amidated peptide is assembled on 4-
20 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-

protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3680.1

EXAMPLE 80

10

Preparation of Peptide having SEQ ID NO. 73

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
15 Ser-NH₂ [SEQ. ID. NO. 73]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the

lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3623.0.

EXAMPLE 81

5 Preparation of Peptide having SEQ ID NO. 74

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser-
NH₂ [SEQ. ID. NO. 74]

The above-identified amidated peptide is assembled on 4-
10 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Used in analysis are Solvent A (0.1% TFA in water) and
15 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3593.0

20

EXAMPLE 82

Preparation of Peptide having SEQ ID NO. 75

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser-
NH₂ [SEQ. ID. NO. 75]

The above-identified amidated peptide is assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Used in analysis are Solvent A (0.1% TFA in water) and
10 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3535.9

15

EXAMPLE 83Preparation of Peptide having SEQ ID NO. 76

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro-NH₂
[SEQ. ID. NO. 76]

20

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and
5 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3505.94.

10

EXAMPLE 84Preparation of Peptide having SEQ ID NO. 77

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro-NH₂
[SEQ. ID. NO. 77]

15

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
20 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%

to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3448.8.

5

EXAMPLE 85Preparation of Peptide having SEQ ID NO. 78

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly-NH₂ [SEQ.
ID. NO. 78]

10

The above-identified peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M):

20 calculated 3351.7.

EXAMPLE 86

Preparation of Peptide having SEQ ID NO. 79

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly-NH₂ [SEQ. ID.
NO. 79]

- 5 The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,
deprotected and purified in a similar way to Example 46. Used
10 in analysis are Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide is then carried out to determine the retention time of
the product peptide. Electrospray Mass Spectrometry (M):
15 calculated 3351.8.

EXAMPLE 87Preparation of Peptide having SEQ ID NO. 80

- His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly-NH₂ [SEQ. ID.
20 NO. 80]

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 46. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
10 Spectrometry (M): calculated 3294.7.

EXAMPLE 88

Preparation of Peptide having SEQ ID NO. 81

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly tPro Ser
15 Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 81]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
20 the resin, deprotected and purified in a similar way to Example
46. Double couplings are required at residues 37,36 and 31.

Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M):
5 calculated 4197.1.

EXAMPLE 89

Preparation of Peptide having SEQ ID NO. 82

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 82]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
15 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Double couplings are required at residues 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
20 Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of

the product peptide. Electrospray Mass Spectrometry (M):
calculated 4179.1.

EXAMPLE 90

Preparation of Peptide having SEQ ID NO. 83

5 His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly NMeala
Ser Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 83]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Double couplings are required at residues 36 and 31. Used
in analysis are Solvent A (0.1% TFA in water) and Solvent B
15 (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide is then carried out to determine the retention time of
the product peptide. Electrospray Mass Spectrometry (M):
calculated 3948.3.

20

EXAMPLE 91

Preparation of Peptide having SEQ ID NO. 84

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly NMeala
Ser Ser Gly Ala NMeala Nmeala-NH₂ [SEQ. ID. NO. 84]

The above-identified amidated peptide is assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Double couplings are required at residues 36 and 31. Used
10 in analysis are Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide is then carried out to determine the retention time of
the product peptide. Electrospray Mass Spectrometry (M):
15 calculated 3840.1.

EXAMPLE 92

Preparation of Peptide having SEQ ID NO. 85

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly hPro Ser
20 Ser Gly Ala hPro hPro-NH₂ [SEQ. ID. NO. 85]

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 46. Double couplings are required at residues 36 and 31. Used
in analysis are Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide is then carried out to determine the retention time of
10 the product peptide. Electrospray Mass Spectrometry (M):
calculated 4050.1.

EXAMPLE 93

Preparation of Peptide having SEQ ID NO. 86

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly hPro Ser
Ser Gly Ala hPro-NH₂ [SEQ. ID. NO. 86]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
20 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example

46. A double coupling is required at residue 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then
5 carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3937.1

EXAMPLE 94

Preparation of Peptide having SEQ ID NO. 87

10 Arg Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala-NH₂ [SEQ. ID. NO. 87]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
15 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
20 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the

retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3827.2.

EXAMPLE 95

Preparation of Peptide having SEQ ID NO. 88

5 His Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-NH₂ [SEQ.
ID. NO. 88]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
15 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3394.8.

EXAMPLE 96

20 Preparation of Peptide having SEQ ID NO. 89

His Gly Glu Gly Thr Naphthylala Thr Ser Asp Leu Ser Lys Gln Leu

Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂

[SEQ. ID. NO. 89]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
10 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3289.5.

EXAMPLE 97

15 Preparation of Peptide having SEQ ID NO. 90

His Gly Glu Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
90]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-

protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3280.7.

EXAMPLE 98

Preparation of Peptide having SEQ ID NO. 91

His Gly Glu Gly Thr Phe Ser Thr Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 91]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the

lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3294.7.

EXAMPLE 99

5 Preparation of Peptide having SEQ ID NO. 92

His Gly Glu Gly Thr Phe Thr Ser Glu Leu Ser Lys Gln Met Ala Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
92]

The above-identified amidated peptide is assembled on 4-
10 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Used in analysis are Solvent A (0.1% TFA in water) and
15 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3250.7.

20

EXAMPLE 100

Preparation of Peptide having SEQ ID NO. 93

His Gly Glu Gly Thr Phe Thr Ser Asp pentylgly Ser Lys Gln Leu
Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 93]

The above-identified amidated peptide is assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
46. Used in analysis are Solvent A (0.1% TFA in water) and
10 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3253.5.

15

EXAMPLE 101Preparation of Peptide having SEQ ID NO. 94

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Naphthylala Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 94]

20

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and
5 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3289.5.

10

EXAMPLE 102Preparation of Peptide having SEQ ID NO. 95

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe tButylgly Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 95]

15

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
20 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%

to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3183.4.

5

EXAMPLE 103Preparation of Peptide having SEQ ID NO. 96

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Asp Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
96]

10

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example

15

46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

20

Spectrometry (M): calculated 3237.6.

EXAMPLE 104

Preparation of Peptide having SEQ ID NO. 97

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser-NH₂ [SEQ. ID. NO. 97]

- 5 The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
10 46. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
15 Spectrometry (M): calculated 3637.9.

EXAMPLE 105Preparation of Peptide having SEQ ID NO. 98

- His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly-NH₂ [SEQ. ID.
20 NO. 98]

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 46. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
10 Spectrometry (M): calculated 3309.7.

EXAMPLE 106

Preparation of Peptide having SEQ ID NO. 99

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly hPro Ser
15 Ser Gly Ala hPro hPro-NH₂ [SEQ. ID. NO. 99]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
20 the resin, deprotected and purified in a similar way to Example
46. Double couplings are required at residues 36 and 31. Used

in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3711.1.

EXAMPLE 107

Preparation of C-terminal carboxylic acid peptides corresponding to the above C-terminal amide sequences for SEQ ID NOS. 7, 40-

10 61, 68-75, 78-80 and 87-98

Peptides having the sequences of SEQ ID NOS. 7, 40-61, 68-75, 78-80 and 87-98 are assembled on the so called Wang resin (p-alkoxybenzylalcohol resin (Bachem, 0.54 mmole/g)) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry provides an experimentally determined (M).

EXAMPLE 108

Preparation of C-terminal carboxylic acid peptides corresponding to the above C-terminal amide sequences for SEQ ID NOS. 62-67,

76, 77, 81-86 and 99

- 5 Peptides having the sequences of SEQ ID NOS. 62-67, 76, 77, 81-86 and 99 are assembled on the 2-chlorotritylchloride resin (200-400 mesh), 2% DVB (Novabiochem, 0.4-1.0 mmole/g)) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to
- 10 Example 46. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass
- 15 Spectrometry provides an experimentally determined (M).

EXAMPLE 109

Preparation of Peptide having SEQ ID NO. 100

- Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
20 100]

The above amidated peptide was assembled on 4-(2'-4'-

dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.). In general, single-coupling cycles were used throughout the synthesis and Fast Moc (HBTU activation) chemistry was employed. Deprotection (Fmoc group removal) of the growing peptide chain was achieved using piperidine. Final deprotection of the completed peptide resin was achieved using a mixture of triethylsilane (0.2 mL), ethanedithiol (0.2 mL), anisole (0.2 mL), water (0.2 mL) and trifluoroacetic acid (15 mL) according to standard methods (Introduction to Cleavage Techniques, Applied Biosystems, Inc.) The peptide was precipitated in ether/water (50 mL) and centrifuged. The precipitate was reconstituted in glacial acetic acid and lyophilized. The lyophilized peptide was dissolved in water). Crude purity was about 75%.

Used in purification steps and analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN).

The solution containing peptide was applied to a preparative C-18 column and purified (10% to 40% Solvent B in Solvent A over 40 minutes). Purity of fractions was determined isocratically using a C-18 analytical column. Pure fractions

were pooled furnishing the above-identified peptide. Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 19.2 minutes. Electrospray Mass Spectrometry (M): calculated 3171.6; found 3172.

EXAMPLE 110

Preparation of Peptide having SEQ ID NO. 101

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
10 101]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 36% to 46% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time of 14.9 minutes. Electrospray Mass Spectrometry (M): calculated 3179.6; found 3180.

EXAMPLE 111Preparation of Peptide having SEQ ID NO. 102

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.

5 102]

The above amidated peptide was assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine
MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected
amino acids (Applied Biosystems, Inc.), cleaved from the resin,
10 deprotected and purified in a similar way to Example 109. Used
in analysis were Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 37% to 47%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide gave product peptide having an observed retention time
15 of 12.2 minutes. Electrospray Mass Spectrometry (M): calculated
3251.6; found 3253.3.

EXAMPLE 112Preparation of Peptide having SEQ ID NO. 103

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Leu Glu Glu
20 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
103]

The above amidated peptide was assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, 5 deprotected and purified in a similar way to Example 109. Used in analysis were Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 35% to 45% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide gave product peptide having an observed retention time 10 of 16.3 minutes. Electrospray Mass Spectrometry (M): calculated 3193.6; found 3197.

EXAMPLE 113

Preparation of Peptide having SEQ ID NO. 104

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 104]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc- 20 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example

109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
5 retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3228.6.

EXAMPLE 114

Preparation of Peptide having SEQ ID NO. 105

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
105]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
15 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
20 lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass

Spectrometry (M): calculated 3234.7.

EXAMPLE 115

Preparation of Peptide having SEQ ID NO. 106

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
5 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID.
NO. 106]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3308.7.

EXAMPLE 116Preparation of Peptide having SEQ ID NO. 107

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.

5 107]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
15 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3250.7

EXAMPLE 117Preparation of Peptide having SEQ ID NO. 108

His Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu Glu
20 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 108]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3252.6.

EXAMPLE 118

Preparation of Peptide having SEQ ID NO. 109

Ala Ala Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 109]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example

109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3200.6.

EXAMPLE 119

Preparation of Peptide having SEQ ID NO. 110

Ala Ala Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 110]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

Spectrometry (M): calculated 3143.5.

EXAMPLE 120

Preparation of Peptide having SEQ ID NO. 111

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
5 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
111]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
10 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3214.6.

EXAMPLE 121

Preparation of Peptide having SEQ ID NO. 112

20 Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.

112]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3157.5.

EXAMPLE 122

Preparation of Peptide having SEQ ID NO. 113

Ala Gly Asp Gly Ala Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 113]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from

the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3184.6.

EXAMPLE 123

Preparation of Peptide having SEQ ID NO. 114

10 Ala Gly Asp Gly Ala Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
114]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
15 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
20 to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the

retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3127.5.

EXAMPLE 124

Preparation of Peptide having SEQ ID NO. 115

5 Ala Gly Asp Gly Thr NaphthylAla Thr Ser Asp Leu Ser Lys Gln Met
Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 115]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
15 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3266.4.

EXAMPLE 125

20 Preparation of Peptide having SEQ ID NO. 116

Ala Gly Asp Gly Thr NaphthylAla Thr Ser Asp Leu Ser Lys Gln Leu

Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 116]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
10 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3209.4.

EXAMPLE 126

15 Preparation of Peptide having SEQ ID NO. 117

Ala Gly Asp Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
117]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-

protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
5 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3200.6.

EXAMPLE 127

10 Preparation of Peptide having SEQ ID NO. 118

Ala Gly Asp Gly Thr Phe Ser Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
118]

The above-identified amidated peptide is assembled on 4-
15 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and
20 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the

lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3143.5.

EXAMPLE 128

5 Preparation of Peptide having SEQ ID NO. 119

Ala Gly Asp Gly Thr Phe Thr Ala Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
119]

The above-identified amidated peptide is assembled on 4-
10 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
15 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3198.6.

20

EXAMPLE 129

Preparation of Peptide having SEQ ID NO. 120

Ala Gly Asp Gly Thr Phe Thr Ala Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
120]

The above-identified amidated peptide is assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
10 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3141.5.

15

EXAMPLE 130Preparation of Peptide having SEQ ID NO. 121

Ala Gly Asp Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
121]

20

The above-identified peptide is assembled on 4-(2'-4'-
dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine

MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3170.6.

10

EXAMPLE 131Preparation of Peptide having SEQ ID NO. 122

Ala Gly Asp Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 122]

15

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%

20

to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3113.5.

5

EXAMPLE 132Preparation of Peptide having SEQ ID NO. 123

Ala Gly Asp Gly Thr Phe Thr Ser Glu Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
123]

10

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example

15

109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass

20

Spectrometry (M): calculated 3228.6.

EXAMPLE 133

Preparation of Peptide having SEQ ID NO. 124

Ala Gly Asp Gly Thr Phe Thr Ser Glu Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
124]

- 5 The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
10 109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
15 Spectrometry (M): calculated 3171.6.

EXAMPLE 134Preparation of Peptide having SEQ ID NO. 125

Ala Gly Asp Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.

20 125]

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
10 Spectrometry (M): calculated 3172.5.

EXAMPLE 135

Preparation of Peptide having SEQ ID NO. 126

Ala Gly Asp Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
15 126]

The above-identified amidated peptiden is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
20 the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and

Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3115.4.

EXAMPLE 136

Preparation of Peptide having SEQ ID NO. 127

Ala Gly Asp Gly Thr Phe Thr Ser Asp Pentylgly Ser Lys Gln Met
Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
10 ID. NO. 127]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
15 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
20 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

Spectrometry (M): calculated 3230.4.

EXAMPLE 137

Preparation of Peptide having SEQ ID NO. 128

Ala Gly Asp Gly Thr Phe Thr Ser Asp Pentylgly Ser Lys Gln Leu
5 Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 128]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3198.6.

EXAMPLE 138Preparation of Peptide having SEQ ID NO. 129

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.

5 129]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
15 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3141.5.

EXAMPLE 139Preparation of Peptide having SEQ ID NO. 130

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ala Lys Gln Leu Glu Glu
20 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 130]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
5 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
10 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3157.5.

EXAMPLE 140

Preparation of Peptide having SEQ ID NO. 131

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 131]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
20 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example

109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3100.4.

EXAMPLE 141

Preparation of Peptide having SEQ ID NO. 132

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Ala Gln Leu Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 132]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

Spectrometry (M): calculated 3157.6.

EXAMPLE 142

Preparation of Peptide having SEQ ID NO. 133

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Ala Met Glu Glu
5 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
133]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
10 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3100.5.

EXAMPLE 143

Preparation of Peptide having SEQ ID NO. 134

20 Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Ala Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.

134]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3100.5.

EXAMPLE 144Preparation of Peptide having SEQ ID NO. 135

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 135]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from

the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3154.5.

EXAMPLE 145

Preparation of Peptide having SEQ ID NO. 136

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Ala Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 136]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the

retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3115.5.

EXAMPLE 146

Preparation of Peptide having SEQ ID NO. 137

5 Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Pentylgly
Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 137]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
15 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3212.4.

EXAMPLE 147

20 Preparation of Peptide having SEQ ID NO. 138

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Pentylgly

Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 138]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
10 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3173.4.

EXAMPLE 148

15 Preparation of Peptide having SEQ ID NO. 139

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Ala Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 139]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
20 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-

protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
5 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3156.6.

EXAMPLE 149

10 Preparation of Peptide having SEQ ID NO. 140

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Ala Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
140]

The above-identified amidated peptide is assembled on 4-
15 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and
20 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the

lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3099.5.

EXAMPLE 150

5 Preparation of Peptide having SEQ ID NO. 141

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Ala
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
141]

The above-identified amidated peptide is assembled on 4-
10 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
15 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3156.6.

20

EXAMPLE 151

Preparation of Peptide having SEQ ID NO. 142

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Ala
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
142]

The above-identified amidated peptide is assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
10 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3099.5.

15

EXAMPLE 152Preparation of Peptide having SEQ ID NO. 143

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Ala Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
143]

20 The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and
5 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3156.6.

10

EXAMPLE 153Preparation of Peptide having SEQ ID NO. 144

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Ala Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
144]

15

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
20 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%

to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3099.5.

5

EXAMPLE 154Preparation of Peptide having SEQ ID NO. 145

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Ala Arg Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
145]

10

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example

15

109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

20 Spectrometry (M): calculated 3186.6.

EXAMPLE 155

Preparation of Peptide having SEQ ID NO. 146

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Ala Arg Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
146]

5 The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
10 109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
15 Spectrometry (M): calculated 3129.5.

EXAMPLE 156Preparation of Peptide having SEQ ID NO. 147

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Ala Leu Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
20 147]

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
10 Spectrometry (M): calculated 3129.5.

EXAMPLE 157

Preparation of Peptide having SEQ ID NO. 148

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
15 Glu Ala Val Ala Leu Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
148]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
20 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example

109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
5 retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3072.4.

EXAMPLE 158

Preparation of Peptide having SEQ ID NO. 149

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
10 Glu Ala Val Arg Ala Phe Ile Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
149]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
15 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
20 lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass

Spectrometry (M): calculated 3172.5.

EXAMPLE 159

Preparation of Peptide having SEQ ID NO. 150

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
5 Glu Ala Val Arg Ala Phe Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
150]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
10 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3115.5.

EXAMPLE 160

Preparation of Peptide having SEQ ID NO. 151

20 Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Naphthylala Ile Glu Trp Leu Lys Asn-NH₂ [SEQ.

ID. NO. 151]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3266.4.

EXAMPLE 161

Preparation of Peptide having SEQ ID NO. 152

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu Glu Ala Val Arg Leu Naphthylala Ile Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO. 152]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from

the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3209.4.

EXAMPLE 162

Preparation of Peptide having SEQ ID NO. 153

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Val Glu Trp Leu Lys Asn-NH₂ [SEQ. ID. NO. 153]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the

retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3200.6.

EXAMPLE 163

Preparation of Peptide having SEQ ID NO. 154

5 Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Val Glu Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
154]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
15 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3143.5.

EXAMPLE 164

20 Preparation of Peptide having SEQ ID NO. 155

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe tButylgly Glu Trp Leu Lys Asn-NH₂ [SEQ.
ID. NO. 155]

The above-identified amidated peptide is assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
10 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3216.5.

15

EXAMPLE 165Preparation of Peptide having SEQ ID NO. 156

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe tButylgly Glu Phe Leu Lys Asn-NH₂ [SEQ.
ID. NO. 156]

20

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and
5 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3159.4.

10

EXAMPLE 166Preparation of Peptide having SEQ ID NO. 157

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Asp Trp Leu Lys Asn-NH₂ [SEQ. ID. NO.
157]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
20 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%

to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3200.6.

5

EXAMPLE 167Preparation of Peptide having SEQ ID NO. 158

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Asp Phe Leu Lys Asn-NH₂ [SEQ. ID. NO.
158]

10

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example

15

109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

20

Spectrometry (M): calculated 3143.5.

EXAMPLE 168

Preparation of Peptide having SEQ ID NO. 159

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn-NH₂ [SEQ. ID. NO.
159]

- 5 The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
10 109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
15 Spectrometry (M): calculated 3099.5.

EXAMPLE 169Preparation of Peptide having SEQ ID NO. 160

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Ala Leu Lys Asn-NH₂ [SEQ. ID. NO.

20 160]

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
10 Spectrometry (M): calculated 3081.4.

EXAMPLE 170

Preparation of Peptide having SEQ ID NO. 161

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Ala Lys Asn-NH₂ [SEQ. ID. NO.
15 161]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
20 the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and

Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass

5 Spectrometry (M): calculated 3172.5.

EXAMPLE 171

Preparation of Peptide having SEQ ID NO. 162

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Ala Lys Asn-NH₂ [SEQ. ID. NO.

10 162]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
15 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
20 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3115.5.

EXAMPLE 172Preparation of Peptide having SEQ ID NO. 163

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Ala Asn-NH₂ [SEQ. ID. NO.

5 163]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
15 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3157.5.

EXAMPLE 173Preparation of Peptide having SEQ ID NO. 164

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
20 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Ala Asn-NH₂ [SEQ. ID. NO.
164]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
5 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
10 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3100.4.

EXAMPLE 174

Preparation of Peptide having SEQ ID NO. 165

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Ala-NH₂ [SEQ. ID. NO. 165]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
20 the resin, deprotected and purified in a similar way to Example

109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
5 retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3171.6.

EXAMPLE 175

Preparation of Peptide having SEQ ID NO. 166

Ala Gly Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Ala-NH₂ [SEQ. ID. NO.
166]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
15 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
20 lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass

Spectrometry (M): calculated 3114.5.

EXAMPLE 176

Preparation of Peptide having SEQ ID NO. 167

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
5 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 167]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
10 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the
15 lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4033.5.

EXAMPLE 177

Preparation of Peptide having SEQ ID NO. 168

20 His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser

Ser Gly Ala Pro Pro Pro-NH₂ [SEQ. ID. NO. 168]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3984.4.

EXAMPLE 178

Preparation of Peptide having SEQ ID NO. 169

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser Ser Gly Ala Pro Pro-NH₂ [SEQ. ID. NO. 169]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from

the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4016.5.

EXAMPLE 179

Preparation of Peptide having SEQ ID NO. 170

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 170]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the

retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3861.3.

EXAMPLE 180

Preparation of Peptide having SEQ ID NO. 171

5 Ala Gly Glu Gly Thr Phe Thr Ser Asp Ala Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro-NH₂ [SEQ. ID. NO. 171]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
10 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
15 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3746.1.

EXAMPLE 181

20 Preparation of Peptide having SEQ ID NO. 172

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu

Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala-NH₂ [SEQ. ID. NO. 172]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
5 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
10 to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3742.1.

EXAMPLE 182

15 Preparation of Peptide having SEQ ID NO. 173

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala-NH₂ [SEQ. ID. NO. 173]

The above-identified amidated peptide is assembled on 4-
20 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-

protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
5 to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3693.1.

EXAMPLE 183

10 Preparation of Peptide having SEQ ID NO. 174

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly-NH₂ [SEQ. ID. NO. 174].

The above-identified amidated peptide is assembled on 4-
15 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and
20 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the

lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3751.2.

EXAMPLE 184

5 Preparation of Peptide having SEQ ID NO. 175

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser-NH₂ [SEQ. ID. NO. 175]

The above-identified amidated peptide is assembled on 4-
10 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
15 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3634.1.

20

EXAMPLE 185

Preparation of Peptide having SEQ ID NO. 176

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser-
NH₂ [SEQ. ID. NO. 176]

The above-identified amidated peptide is assembled on 4-
5 (2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
10 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 3526.9.

15

EXAMPLE 186Preparation of Peptide having SEQ ID NO. 177

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser-
NH₂ [SEQ. ID. NO. 177]

20

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide

norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and
5 Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3477.9.

10

EXAMPLE 187Preparation of Peptide having SEQ ID NO. 178

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro-NH₂
[SEQ. ID. NO. 178]

15 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
20 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%

to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3519.9.

5

EXAMPLE 188Preparation of Peptide having SEQ ID NO. 179

His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly-NH₂ [SEQ.
ID. NO. 179]

10

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 15 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass 20 Spectrometry (M): calculated 3307.7.

EXAMPLE 189

Preparation of Peptide having SEQ ID NO. 180

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly-NH₂ [SEQ. ID.
NO. 180]

- 5 The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
10 109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
retention time of the product peptide. Electrospray Mass
15 Spectrometry (M): calculated 3186.5.

EXAMPLE 190Preparation of Peptide having SEQ ID NO. 181

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly tPro Ser
20 Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 181]

The above-identified amidated peptide is assembled on 4-

(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
5 109. Double couplings are required at residues 37,36 and 31.
Used in analysis are Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide is then carried out to determine the retention time of
10 the product peptide. Electrospray Mass Spectrometry (M):
calculated 4121.1.

EXAMPLE 191

Preparation of Peptide having SEQ ID NO. 182

His Gly Glu Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala tPro tPro tPro-NH₂ [SEQ. ID. NO. 182].

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
20 protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example

109. Double couplings are required at residues 37, 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4173.2.

EXAMPLE 192

Preparation of Peptide having SEQ ID NO. 183

10 His Gly Glu Gly Thr Phe Thr Ser Ala Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly NMeala
Ser Ser Gly Ala NMeala NMeala-NH₂ [SEQ. ID. NO. 183]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
15 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example 109. Double couplings are required at residues 36 and 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B
20 (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized

peptide is then carried out to determine the retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3796.1.

EXAMPLE 193

5 Preparation of Peptide having SEQ ID NO. 184

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly hPro Ser
Ser Gly Ala hPro-NH₂ [SEQ. ID. NO. 184]

10 The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example
15 109. A double coupling is required at residue 31. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the retention time of the product
20 peptide. Electrospray Mass Spectrometry (M): calculated 3871.1.

EXAMPLE 194Preparation of Peptide having SEQ ID NO. 185

His Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
5 Ser Gly Ala-NH₂ [SEQ. ID. NO. 185]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
10 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
15 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3750.2.

EXAMPLE 195Preparation of Peptide having SEQ ID NO. 186

His Gly Asp Ala Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
20 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly-NH₂ [SEQ. ID. NO. 186]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-protected amino acids (Applied Biosystems, Inc.), cleaved from
5 the resin, deprotected and purified in a similar way to Example 109. Used in analysis are Solvent A (0.1% TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60% Solvent B in Solvent A over 30 minutes) of the lyophilized peptide is then carried out to determine the
10 retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 3408.8.

EXAMPLE 196

Preparation of Peptide having SEQ ID NO. 187

Ala Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
15 Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 187]

The above-identified amidated peptide is assembled on 4-(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
20 protected amino acids (Applied Biosystems, Inc.), cleaved from the resin, deprotected and purified in a similar way to Example

109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the
5 retention time of the product peptide. Electrospray Mass
Spectrometry (M): calculated 4120.6.

EXAMPLE 197

Preparation of Peptide having SEQ ID NO. 188

Ala Gly Ala Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Leu Glu Glu
10 Glu Ala Val Arg Leu Phe Ile Glu Phe Leu Lys Asn Gly Gly Pro Ser
Ser Gly Ala Pro Pro Pro Ser-NH₂ [SEQ. ID. NO. 188]

The above-identified amidated peptide is assembled on 4-
(2'-4'-dimethoxyphenyl)-Fmoc aminomethyl phenoxy acetamide
15 norleucine MBHA resin (Novabiochem, 0.55 mmole/g) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to Example
109. Used in analysis are Solvent A (0.1% TFA in water) and
Solvent B (0.1% TFA in ACN). Analytical RP-HPLC (gradient 30%
20 to 60% Solvent B in Solvent A over 30 minutes) of the
lyophilized peptide is then carried out to determine the

retention time of the product peptide. Electrospray Mass Spectrometry (M): calculated 4005.5.

EXAMPLE 198

Preparation of C-terminal carboxylic acid peptides corresponding

5 to the above C-terminal amide sequences
 for Peptides having SEQ ID NOS. 100-166, 172-177,
 179-180 and 185-188.

C-terminal carboxylic acid peptides corresponding to
amidated having SEQ ID NOS. 100-166, 172-177, 179-180 and 185-
10 188 are assembled on the so called Wang resin (p-
alkoxybenzylalcohol resin (Bachem, 0.54 mmole/g)) using Fmoc-
protected amino acids (Applied Biosystems, Inc.), cleaved from
the resin, deprotected and purified in a similar way to that
described in Example 109. Used in analysis are Solvent A (0.1%
15 TFA in water) and Solvent B (0.1% TFA in ACN). Analytical RP-
HPLC (gradient 30% to 60% Solvent B in Solvent A over 30
minutes) of the lyophilized peptide is then carried out to
determine the retention time of the product peptide.
Electrospray Mass Spectrometry provides an experimentally
20 determined (M).

EXAMPLE 199

Preparation of C-terminal carboxylic acid peptides corresponding
to the above C-terminal amide sequences
for Peptides having SEQ ID NOS. 167-171, 178 and 181-184.

- C-terminal carboxylic acid peptides corresponding to
- 5 amidated SEQ ID NOS. 167-171, 178 and 181-184 are assembled on
the 2-chlorotritylchloride resin (200-400 mesh), 2% DVB
(Novabiochem, 0.4-1.0 mmole/g)) using Fmoc-protected amino acids
(Applied Biosystems, Inc.), cleaved from the resin, deprotected
and purified in a similar way to that described in Example 109.
- 10 Used in analysis are Solvent A (0.1% TFA in water) and Solvent B
(0.1% TFA in ACN). Analytical RP-HPLC (gradient 30% to 60%
Solvent B in Solvent A over 30 minutes) of the lyophilized
peptide is then carried out to determine the retention time of
the product peptide. Electrospray Mass Spectrometry provides an
- 15 experimentally determined (M).

EXAMPLES A TO E

Reagents Used

- GLP-1[7-36]NH₂ (GLP-1) was purchased from Bachem (Torrance,
20 CA). All other peptides were prepared using synthesis methods
such as those described therein. All chemicals were of the

highest commercial grade. The cAMP SPA immunoassay was purchased from Amersham. The radioligands were purchased from New England Nuclear (Boston, MA). RINm5f cells (American Type Tissue Collection, Rockville, MD) were grown in DME/F12 medium
5 containing 10% fetal bovine serum and 2mM L-glutamine. Cells were grown at 37°C and 5% CO₂/95% humidified air and medium was replaced every 2 to 3 days. Cells were grown to confluence then harvested and homogenized using on a Polytron homogenizer. Cell homogenates were stored frozen at -70°C until used.

10

EXAMPLE A - GLP-1 RECEPTOR BINDING STUDIES

Receptor binding was assessed by measuring displacement of [¹²⁵I]GLP-1 or [¹²⁵I]exendin(9-39) from RINm5f membranes. Assay buffer contained 5 µg/ml bestatin, 1 µg/ml phosphoramidon, 1 mg/ml bovine serum albumin (fraction V), 1 mg/ml bacitracin, and
15 1 mM MgCl₂ in 20 mM HEPES, pH 7.4. To measure binding, 30 µg membrane protein (Bradford protein assay) was resuspended in 200 µl assay buffer and incubated with 60 pM [¹²⁵I]GLP-1 or [¹²⁵I]exendin(9-39) and unlabeled peptides for 120 minutes at
23 C in 96 well plates (Nagle Nunc, Rochester, NY). Incubations
20 were terminated by rapid filtration with cold phosphate buffered saline, pH 7.4, through polyethyleneimine-treated GF/B glass

fiber filters (Wallac Inc., Gaithersburg, MD) using a Tomtec Mach II plate harvester (Wallac Inc., Gaithersburg, MD). Filters were dried, combined with scintillant, and radioactivity determined in a Betaplate liquid scintillant counter (Wallac Inc.).

Peptide samples were run in the assay as duplicate points at 6 dilutions over a concentration range of 10^{-6} M to 10^{-12} M to generate response curves. The biological activity of a sample is expressed as an IC_{50} value, calculated from the raw data using an iterative curve-fitting program using a 4-parameter logistic equation (Prizm, GraphPAD Software).

EXAMPLE B - CYCLASE ACTIVATION STUDY

Assay buffer contained 10 μ M GTP, 0.75 mM ATP, 2.5 mM $MgCl_2$, 0.5mM phosphocreatine, 12.5 U/ml creatine kinase, 0.4 mg/ml aprotinin, 1 μ M IBMX in 50 mM HEPES, pH 7.4. Membranes and peptides were combined in 100 μ l of assay buffer in 96 well filter-bottom plates (Millipore Corp., Bedford, MA). After 20 minutes incubation at 37°C, the assay was terminated by transfer of supernatant by filtration into a fresh 96 well plate using a Millipore vacuum manifold. Supernatant cAMP contents were quantitated by SPA immunoassay. Peptide samples were run in the

assay as triplicate points at 7 dilutions over a concentration range of 10^{-6} M to 10^{-12} M to generate response curves. The biological activity of a particular sample was expressed as an EC_{50} value, calculated as described above.

5

EXAMPLE C - DETERMINATION OF
BLOOD GLUCOSE LEVELS IN DB/DB MICE

C57BLKS/J-m-db mice at least 3 months of age were utilized for the study. The mice were obtained from The Jackson Laboratory and allowed to acclimate for at least one week before
10 use. Mice were housed in groups of ten at $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a 12:12 light:dark cycle, with lights on at 6 a.m. All animals were deprived of food for 2 hours before taking baseline blood samples. Approximately 70 μl of blood was drawn from each mouse via eye puncture, after a light anesthesia with metophane.
15 After collecting baseline blood samples, to measure plasma glucose concentrations, all animals receive subcutaneous injections of either vehicle (10.9% NaCl), exendin-4 or test compound (1 μg) in vehicle. Blood samples were drawn again, using the same procedure, after exactly one hour from the
20 injections, and plasma glucose concentrations were measured. For each animal, the % change in plasma value, from baseline

value, was calculated.

EXAMPLE D - DOSE RESPONSE DETERMINATION OF

BLOOD GLUCOSE LEVELS IN DB/DB MICE

C57BLKS/J-m-db/db mice, at least 3 months of age were
5 utilized for the study. The mice were obtained from The Jackson
Laboratory and allowed to acclimate for at least one week before
use. Mice were housed in groups of ten at $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with a
12:12 light:dark cycle, with lights on at 6 a.m. All animals
were deprived of food for 2 hours before taking baseline blood
10 samples. Approximately 70 μl of blood was drawn from each mouse
via eye puncture, after a light anesthesia with metophane.
After collecting baseline blood samples, to measure plasma
glucose concentrations, all animals receive subcutaneous
injections of either vehicle, exendin-4 or test compound in
15 concentrations indicated. Blood samples were drawn again, using
the same procedure, after exactly one hour from the injections,
and plasma glucose concentrations were measured. For each
animal, the % change in plasma value, from baseline value, was
calculated and a dose dependent relationship was evaluated using
20 Graphpad Prizm™ software.

EXAMPLE E - GASTRIC EMPTYING

The following study was and may be carried out to examine the effects of exendin-4 and/or an exendin agonist compound on gastric emptying in rats. This experiment followed a modification of the method of Scarpignato, et al., Arch. Int. Pharmacodyn. Ther. 246:286-94, 1980. Male Harlan Sprague Dawley (HSD) rats were used. All animals were housed at 22.7 ± 0.8 C in a 12:12 hour light:dark cycle (experiments being performed during the light cycle) and were fed and watered *ad libitum* (Diet LM-485, Teklad, Madison, WI). Exendin-4 was synthesized according to standard peptide synthesis methods. The preparation of exendin-4 is described in Example 14. The determination of gastric emptying by the method described below was performed after a fast of -20 hours to ensure that the stomach contained no chyme that would interfere with spectrophotometric absorbance measurements.

Conscious rats received by gavage, 1.5ml of an acacloric gel containing 1.5% methyl cellulose (M-0262, Sigma Chemical Co, St Louis, MO) and 0.05% phenol red indicator. Twenty minutes after gavage, rats were anesthetized using 5% halothane, the stomach exposed and clamped at the pyloric and lower esophageal sphincters using artery forceps, removed and opened into an

alkaline solution which was made up to a fixed volume. Stomach content was derived from the intensity of the phenol red in the alkaline solution, measured by absorbance at a wavelength of 560 nm. In separate experiments on 7 rats, the stomach and
5 small intestine were both excised and opened into an alkaline solution. The quantity of phenol red that could be recovered from the upper gastrointestinal tract within 20 minutes of gavage was $89 \pm 4\%$; dye which appeared to bind irrecoverably to the gut luminal surface may have accounted for the balance. To
10 account for a maximal dye recovery of less than 100%, percent of stomach contents remaining after 20 min were expressed as a fraction of the gastric contents recovered from control rats sacrificed immediately after gavage in the same experiment.

Percent gastric contents remaining = (absorbance at 20
15 min)/(absorbance at 0 min) x 100.

Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the following claims.

WE CLAIM:

1. A pharmaceutical formulation comprising an exendin or an exendin agonist peptide, a buffer, and an iso-osmolality modifier, said pharmaceutical formulation having a pH of between
5 about 3.0 and about 7.0.
2. A pharmaceutical formulation according to claim 1 wherein said buffer is an acetate buffer.
3. A pharmaceutical formulation according to claim 1 wherein said iso-osmolality modifier is mannitol.
- 10 4. A pharmaceutical formulation according to claim 1 wherein said pH is between about 4.0 and about 6.0.
5. A pharmaceutical formulation according to claim 1 wherein said pH is between about 4.0 and about 5.0.
6. A pharmaceutical formulation according to claim 1,
15 further comprising a preservative.
7. A pharmaceutical formulation according to claim 5 wherein said preservative is m-cresol.
8. A pharmaceutical formulation comprising an exendin or an exendin agonist peptide, an acetate buffer, and mannitol,
20 said pharmaceutical formulation having a pH of between about 3.0 and about 7.0.

9. A pharmaceutical formulation according to claim 7, further comprising m-cresol.

10. A pharmaceutical formulation according to claim 8, wherein said pH is between about 4.0 and about 6.0.

5 11. A pharmaceutical formulation according to claim 8, wherein said pH is between about 4.0 and about 5.0.

12. A pharmaceutical formulation according to any of claims 1-11, wherein said pharmaceutical formulation is a liquid.

10 13. A pharmaceutical formulation according to any of claims 1-11, wherein said pharmaceutical formulation is lyophilized.

14. A parenteral liquid pharmaceutical formulation, comprising about 0.005% to about 0.4% (w/v) of an exendin or an
15 exendin agonist peptide in an aqueous system, about 0.02% to 0.5% (w/v) of an acetate, phosphate, citrate, or glutamate buffer, about 1.0% to about 10% (w/v) of a carbohydrate or polyhydric alcohol iso-osmolality modifier (preferably mannitol) said formulation having a pH of between about 3.0 and about 7.0.

20 15. The parenteral liquid pharmaceutical formulation according to claim 14 wherein said formulation comprises from

about 0.005 to about 0.05% (w/v) of an exendin or an exendin agonist peptide.

16. The parenteral liquid pharmaceutical formulation according to claim 14 wherein said formulation comprises from
5 about 0.005 to about 0.02% (w/v) of an exendin or an exendin agonist peptide.

17. The parenteral liquid pharmaceutical formulation according to claim 14 wherein said polyhydric alcohol is selected from the group consisting of sorbitol, mannitol,
10 inositol, glycerol, xylitol, and polyethylene glycols.

18. The parenteral liquid pharmaceutical formulation according to claim 17 wherein said polyhydric alcohol is mannitol.

19. The parenteral liquid pharmaceutical formulation
15 according to claim 14 wherein said carbohydrate is selected from the group consisting of galactose, arabinose, and lactose.

20. The parenteral liquid pharmaceutical formulation according to claim 14 which is an isotonic or iso-osmolar solution in an aqueous continuous phase.

20 21. The parenteral liquid pharmaceutical formulation according to claim 14 wherein said pH is between about 4.0 and

about 6.0.

22. A parenteral liquid pharmaceutical formulation according to claim 14 wherein said pH is between about 4.0 to 5.0.

5 23. The parenteral liquid pharmaceutical formulation according to claim 14, further comprising from about 0.005% to 1.0% (w/v) of an anti-microbial preservative.

24. The parenteral liquid pharmaceutical formulation according to claim 23 wherein said anti-microbial preservative
10 is selected from the group consisting of m-cresol, benzyl alcohol, methyl, ethyl, propyl parabens, butyl parabens, and phenol.

25. The parenteral liquid pharmaceutical formulation according to claim 24 wherein said anti-microbial preservative
15 is m-cresol.

26. The parenteral liquid pharmaceutical formulation according to claim 14 wherein said carbohydrate or polyhydric alcohol is replaced by up to about 0.9% saline.

27. The parenteral liquid pharmaceutical formulation
20 according to claim 26 which is an isotonic or iso-osmolar solution in an aqueous continuous phase.

28. The formulation according to any of claims 1-11, 14-26 or 27, wherein said formulation is a liquid.

29. The formulation according to any of claims 1-11, 14-26 or 27, wherein said formulation is a lyophilized unit-dose or
5 multi-doses formulation containing a bulking agent.

30. The formulation according to claim 28 wherein said bulking agent is an iso-osmolality modifier.

31. The formulation according to claim 28, further comprising a surfactant.

10 32. The formulation according to claim 29 wherein said surfactant comprises about 0.1% to about 1.0% (w/v) of a non-ionic detergent.

33. The formulation according to claim 30 wherein said surfactant is polysorbate 80.

15 34. A solid or dry powder pharmaceutical formulation comprising from between about 1% to about 100% (w/w) of an exendin or an exendin agonist peptide and, wherein said exendin or exendin agonist peptide is present in an amount that is less than about 100% (w/w), a bulking agent.

20 35. The pharmaceutical formulation according to claim 34 wherein said bulking agent comprises from about 0% to about 99%

(w/w) of a carbohydrate or polyhydric alcohol.

36. The pharmaceutical formulation according to claim 34, further comprising a salt.

37. The pharmaceutical formulation according to claim 34
5 which includes a bulking agent and a salt.

38. The pharmaceutical formulation according to claim 34, further comprising a surfactant.

39. The pharmaceutical formulation according to claim 37 wherein said surfactant comprises about 0.1% to about 1.0% (w/w)
10 of a non-ionic detergent.

40. The pharmaceutical formulation according to claim 38 wherein said surfactant is polysorbate 80.

41. A pharmaceutical formulation comprising up to about 50 mg/ml of an exendin or an exendin agonist in 30mM acetate
15 buffer, and mannitol, said formulation having a pH of about 4.5.

42. The pharmaceutical formulation according to claim 41, further comprising a preservative.

43. A method for administering an exendin or an exendin agonist to a subject in need thereof, comprising injecting said
20 subject with about 0.1 to about 0.5 µg per kilogram of an exendin or an exendin agonist.

44. The method according to claim 43 wherein said injection is administered to said subject from one to three times per day.

45. The method according to claim 44 wherein said
5 injection is administered to said subject two times per day.

46. A method for administering an exendin or an exendin agonist to a subject in need thereof, comprising orally administering to said subject about 500 to about 12,000 µg per day of said exendin or exendin agonist in single or divided
10 doses.

47. The method according to claim 46 wherein from about 500 to about 5,000 µg per day of said exendin or exendin agonist is orally administered.

48. A method for administering an exendin or an exendin
15 agonist to a subject in need thereof, comprising administering about 100 to about 12,000 µg per day of said exendin or exendin agonist to the pulmonary system of said subject in single or divided doses.

49. The method according to claim 48 wherein from about
20 500 to about 1,000 µg per day of said exendin or exendin agonist is administered to the pulmonary system of said subject in

single or divided doses.

50. A method for administering an exendin or an exendin agonist to a subject in need thereof, comprising nasally administering from about 10-1000 to about 1200-12,000 μg per day of said exendin or exendin agonist to said subject in single or divided doses.

51. The method according to claim 50 wherein from about 10 to about 1,200 μg per day of said exendin or exendin agonist is nasally administered.

10 52. A method for administering an exendin or an exendin agonist to a subject in need thereof, comprising the buccal administration of from about 10-1000 to about 1200-12,000 μg per day of said exendin or exendin agonist to said subject in single or divided doses.

15 53. The method according to claim 52 wherein from about 10 to about 1,200 μg per day of said exendin or exendin agonist is administered.

54. A method for administering an exendin or an exendin agonist to a subject in need thereof, comprising the sublingual administration of from about 10-1000 to about 1200-8,000 μg per day of said exendin or exendin agonist to said subject in single

or divided doses.

55. The method according to claim 54 wherein from about 10 to about 1,200 μg per day of said exendin or exendin agonist is administered.

5 56. A method for administering an exendin or an exendin agonist to a subject in need thereof, comprising injecting said subject with about 1 μg -30 μg to about 1 mg of an exendin or exendin agonist per day.

57. The method according to claim 56 wherein said
10 injection is a peripheral injection.

58. The method according to claim 56 wherein said subject is injected with about 1-30 μg to about 500 μg of said exendin or exendin agonist per day.

59. The method according to claim 56 wherein said subject
15 is injected with about 1-30 μg to about 50 μg of said exendin or exendin agonist per day.

60. The method according to claim 56 wherein said subject is injected with about 3 μg to about 50 μg of said exendin or exendin agonist per day.

20 61. A method for administering an exendin or an exendin agonist to a subject in need thereof, comprising injecting an

exendin or an exendin agonist into said subject in an amount equal to from about 0.005 µg/kg per dose to about 0.2 µg/kg per dose.

62. The method according to claim 61 wherein said dose is
5 from about 0.02 µg/kg per dose to about 0.1 µg/kg per dose.

63. The method according to claim 61 wherein said dose is from about 0.05 µg/kg per dose to about 0.1 µg/kg per dose.

64. The method according to any of claims 61, 62 or 63,
wherein said doses are administered to said subject from 1 to 4
10 times per day.

65. The method according to any of claims 61, 62 or 63,
wherein said doses are administered to said subject from 1 to 2
times per day.

66. A method for increasing the sensitivity of a a subject
15 to exogenous or endogenous insulin, comprising administering an effective amount of exendin or an exendin agonist to said subject.

67. The method according to claim 66 wherein said exendin or an exendin agonist is administered by nasal administration.

20 68. The method according to claim 66 wherein said exendin or an exendin agonist is administered by oral administration.

69. The method according to claim 66 wherein said exendin or an exendin agonist is administered by pulmonary administration.

70. The method according to claim 66 wherein said exendin
5 or an exendin agonist is administered by buccal administration.

71. The method according to claim 66 wherein said exendin or an exendin agonist is administered by sublingual administration.

72. The method according to claim 66 wherein said exendin
10 or an exendin agonist is administered by intra-tracheal administration.

73. The method according to claim 66 wherein said exendin or an exendin agonist is administered by injection.

74. The method according to claim 73 wherein said
15 injection is a subcutaneous injection.

EXENDIN-3

His Ser Asp Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
1 5 10 15
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
20 25 30
Ser Gly Ala Pro Pro Ser-NH₂
35

FIGURE 1

EXENDIN-4

His Gly Glu Gly Thr Phe Thr Ser Asp Leu Ser Lys Gln Met Glu Glu
5
Glu Ala Val Arg Leu Phe Ile Glu Trp Leu Lys Asn Gly Gly Pro Ser
20
Ser Gly Ala Pro Pro Ser-NH₂
35

FIGURE 2

GLP-1 (GLP-1 [7-36]NH₂)

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly
5
Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg-NH₂
20 30

FIGURE 3

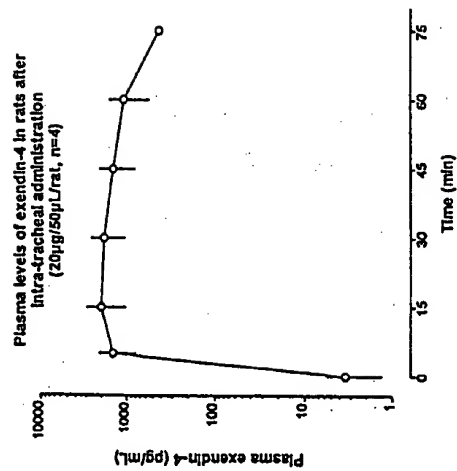
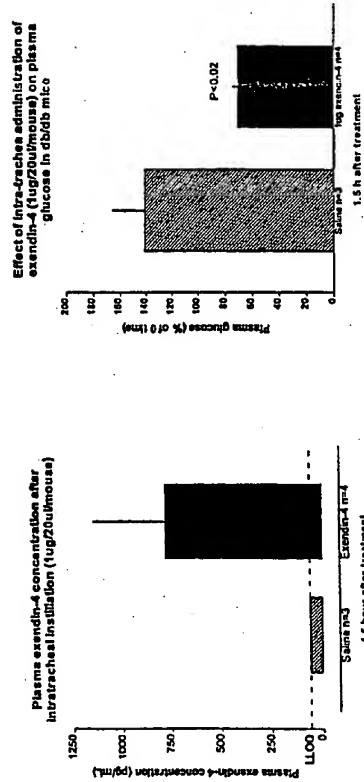
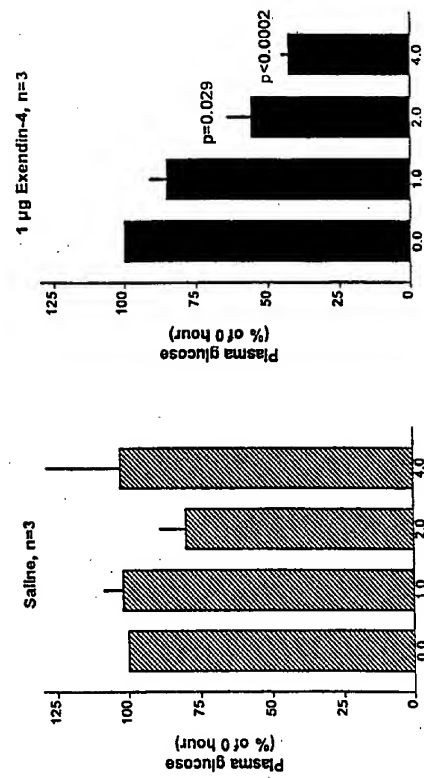


Figure 1. Male rats (350-400g) fasted overnight were cannulated in the trachea and femoral artery under anesthesia. Blood was drawn from the arterial line before and after (5, 15, 30, 45, 60 and 75 min) 20µg of exendin-4 dissolved in 50µL saline was administered into the trachea of each rat. Plasma exendin-4 levels were determined with an immunoradiometric assay.



5a and 5b
Figure A. Male db/db mice (approx 50g) were fasted for 2h, and the trachea was intubated under anesthesia. The animals were bled (75 µL, orbital sinus) before and after 20 µL of saline or 1 µg exendin-4 dissolved in saline was administered into the trachea of each animal.



6/18

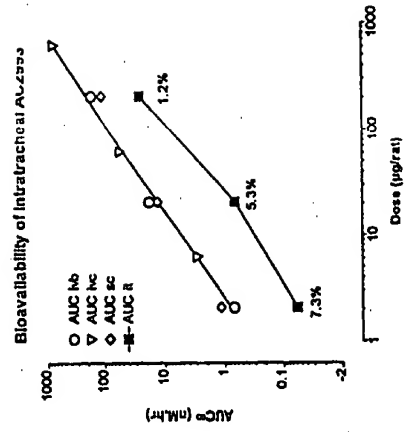


Figure 7b.

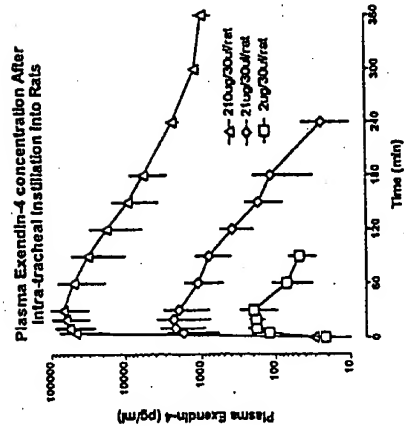


Figure 7a.

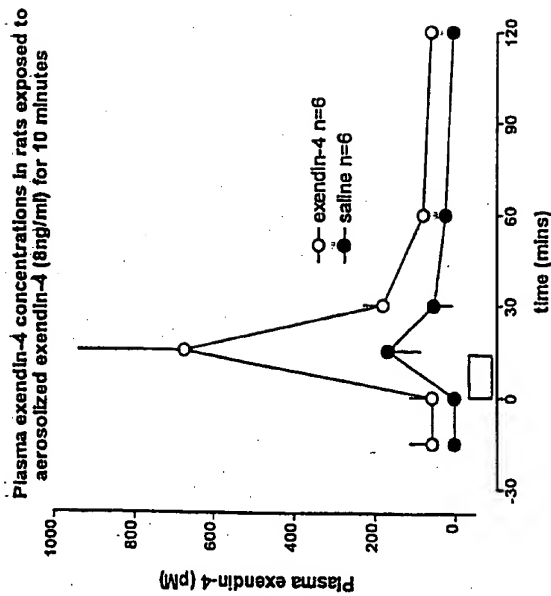
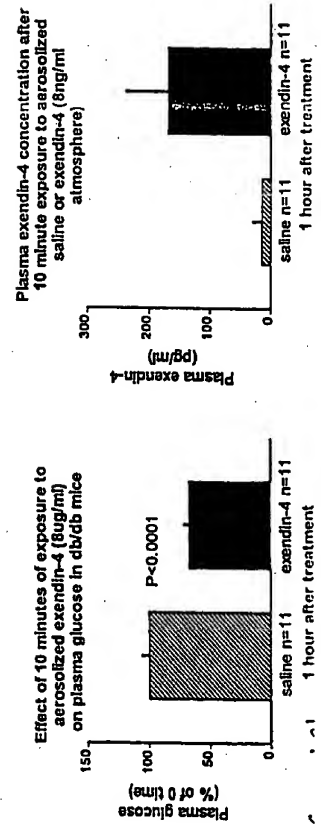
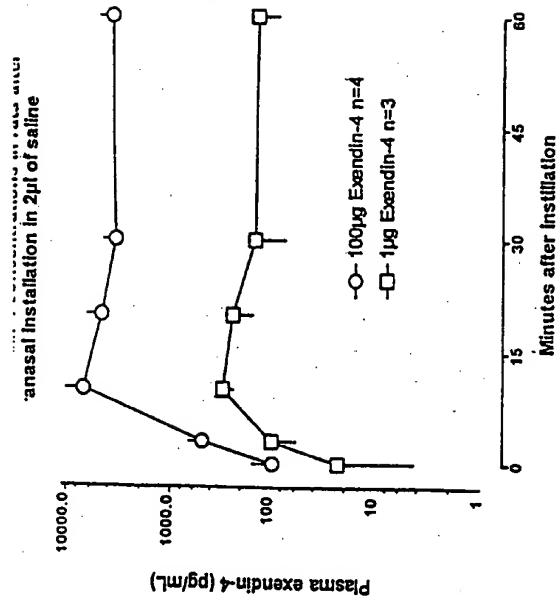


Figure 8. Male rats (approximately 350g each) fasted overnight were placed in a 2 litre chamber and exposed to aerosolized exendin-4 for 10 minutes. Exendin-4 was nebulized at a rate of 0.2mg/min at a flow rate of 5L/min. The concentration of aerosolized exendin-4 was estimated from samples of chamber atmosphere drawn during the course of the experiment.





10
Figure 1. Harlan Sprague Dawley rats 311-365g, nonfasted, were dosed with 0, 1, 100µg of exendin-4 in 2µl of saline by application into the nostrils. Blood samples from anesthetized (Hurricane) tail tip were collected at 0, 3, 10, 20, 30 and 60 min after dosing for exendin-4 plasma level measured by IRMA.

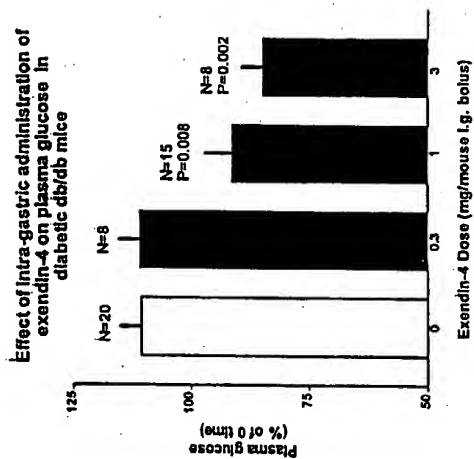
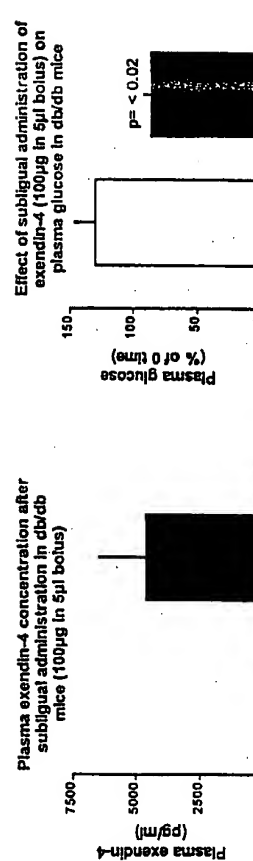


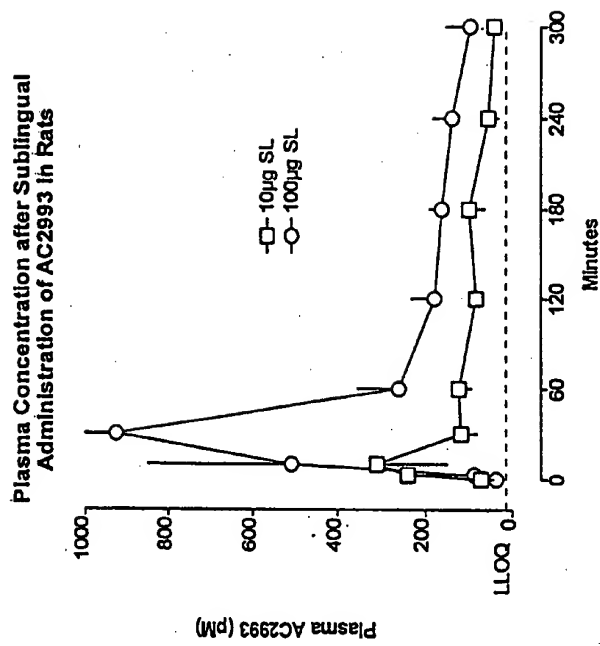
Figure 7 Male db/db mice (approx 50g) were fasted for 2h and bled (40 μ l, orbital sinus) before and 1h after 200 μ l of saline or exendin-4 dissolved in saline was administered i.g. into each animal.

Sublingual

Sublingual application of exendin-4 (100 μ g/5 μ L/animal) to diabetic db/db mice led to a 15% decrease in plasma glucose concentration one hour after treatment. A 30% increase was observed for the control group receiving saline. The mean exendin-4 plasma level at 60min was 4520 ± 1846 pg/mL (see Figure 8).



10/18



Dose was given in 3 µL saline under the tongue in HSD rats (~300g) briefly anesthetized with melophane.

Figure 12c.

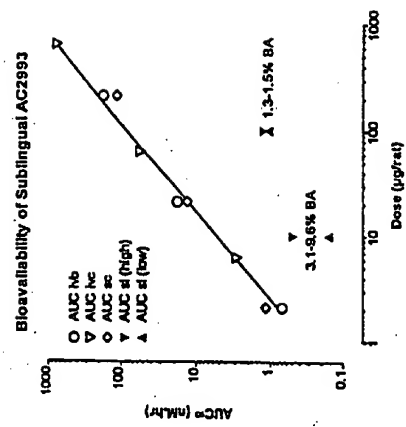


Figure 12d.

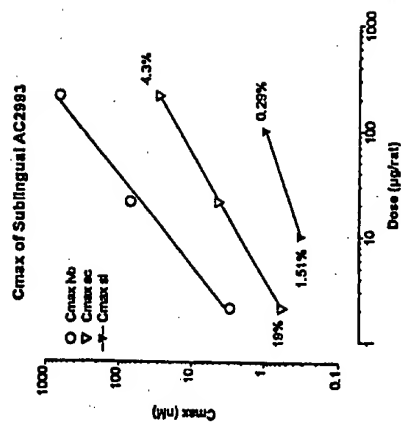
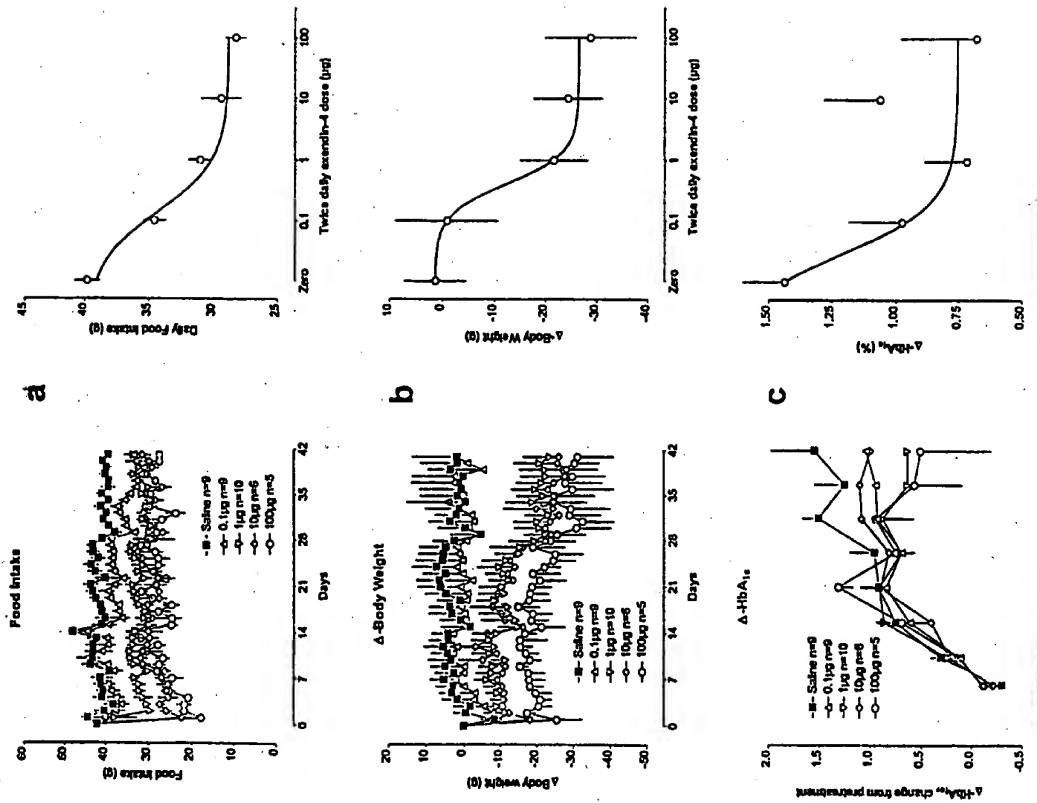


Figure 12e.



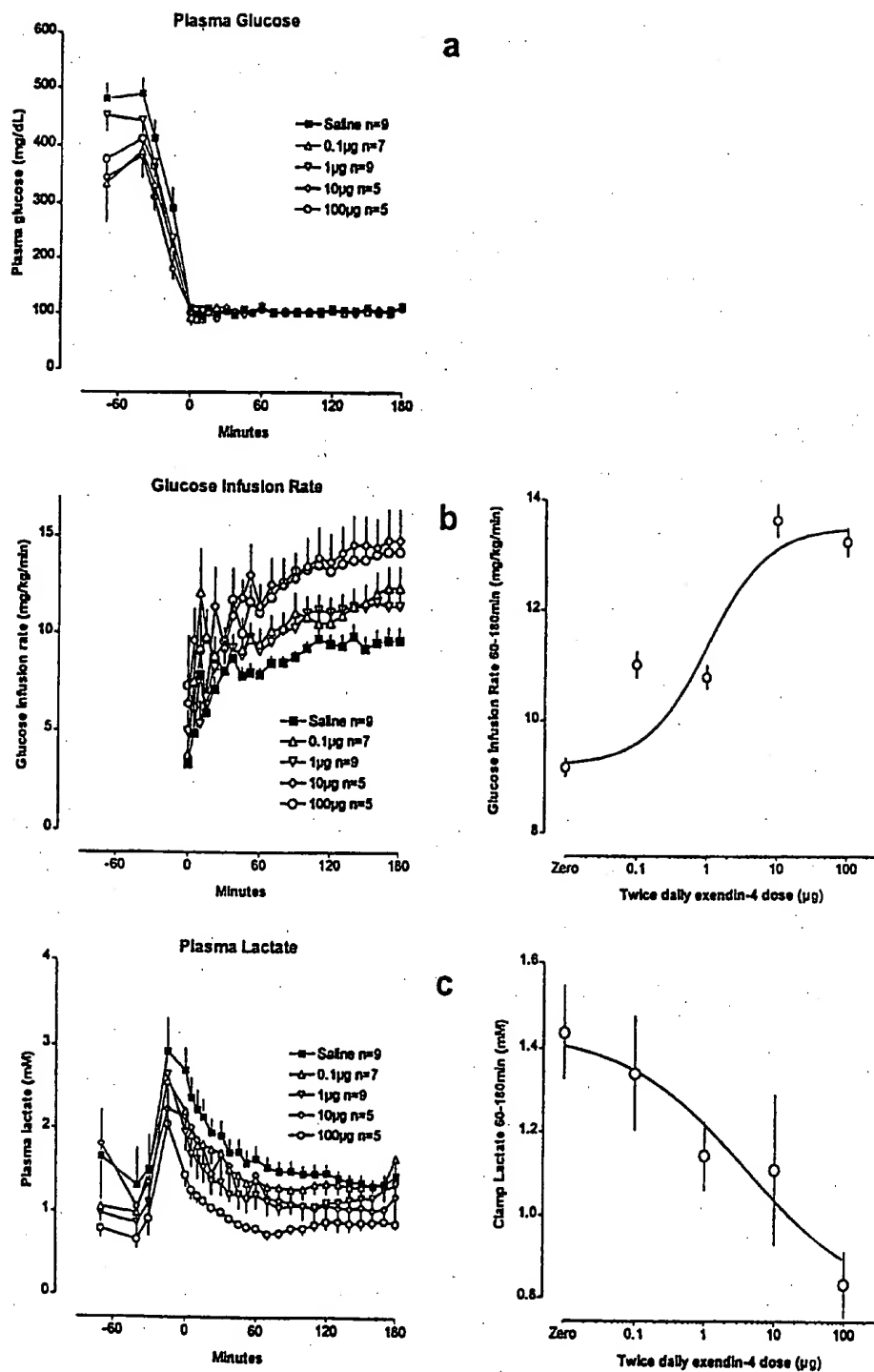


Figure 14

(SEQ. ID. NO.)	Xaa ₁	Xaa ₂	Xaa ₃	Xaa ₄	Xaa ₅	Xaa ₆	Xaa ₇	Xaa ₈	Xaa ₉	Xaa ₁₀	Xaa ₁₁	Xaa ₁₂	Xaa ₁₃	Xaa ₁₄	Xaa ₁₅	Xaa ₁₆	Xaa ₁₇	Xaa ₁₈	Xaa ₁₉
23	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	pGly	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Pro	Ser
24	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	naph	Ile	Glu	Trp	Pro	Pro	Pro	Pro	Pro	Ser
25	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Val	Glu	Trp	Pro	Pro	Pro	Pro	Pro	Ser
26	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Val	Glu	Phe	Pro	Pro	Pro	Pro	Pro	Ser
27	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	tBuG	Glu	Trp	Pro	Pro	Pro	Pro	Pro	Ser
28	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	tBuG	Glu	Phe	Pro	Pro	Pro	Pro	Pro	Ser
29	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Asp	Trp	Pro	Pro	Pro	Pro	Pro	Ser
30	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Phe	Pro	Pro	Pro	Pro	Pro	Ser
31	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	tPro	tPro	tPro	tPro	tPro	Ser
32	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	tPro	tPro	tPro	tPro	Ser
33	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	hPro	hPro	hPro	hPro	hPro	Ser
34	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	hPro	hPro	hPro	hPro	Ser
35	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	tPro	tPro	tPro	tPro	tPro	Ser
36	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	hPro	hPro	hPro	hPro	hPro	Ser
37	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	MeAla	MeAla	MeAla	MeAla	MeAla	Ser
38	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Met	Phe	Ile	Glu	Trp	Pro	MeAla	MeAla	MeAla	MeAla	Ser
39	His	Gly	Glu	Phe	Thr	Ser	Asp	Leu	Leu	Phe	Ile	Glu	Phe	MeAla	MeAla	MeAla	MeAla	MeAla	Ser

FIGURE 15
(Sheet 2 of 2)

AC2993-104 Blood Glucose Part 2

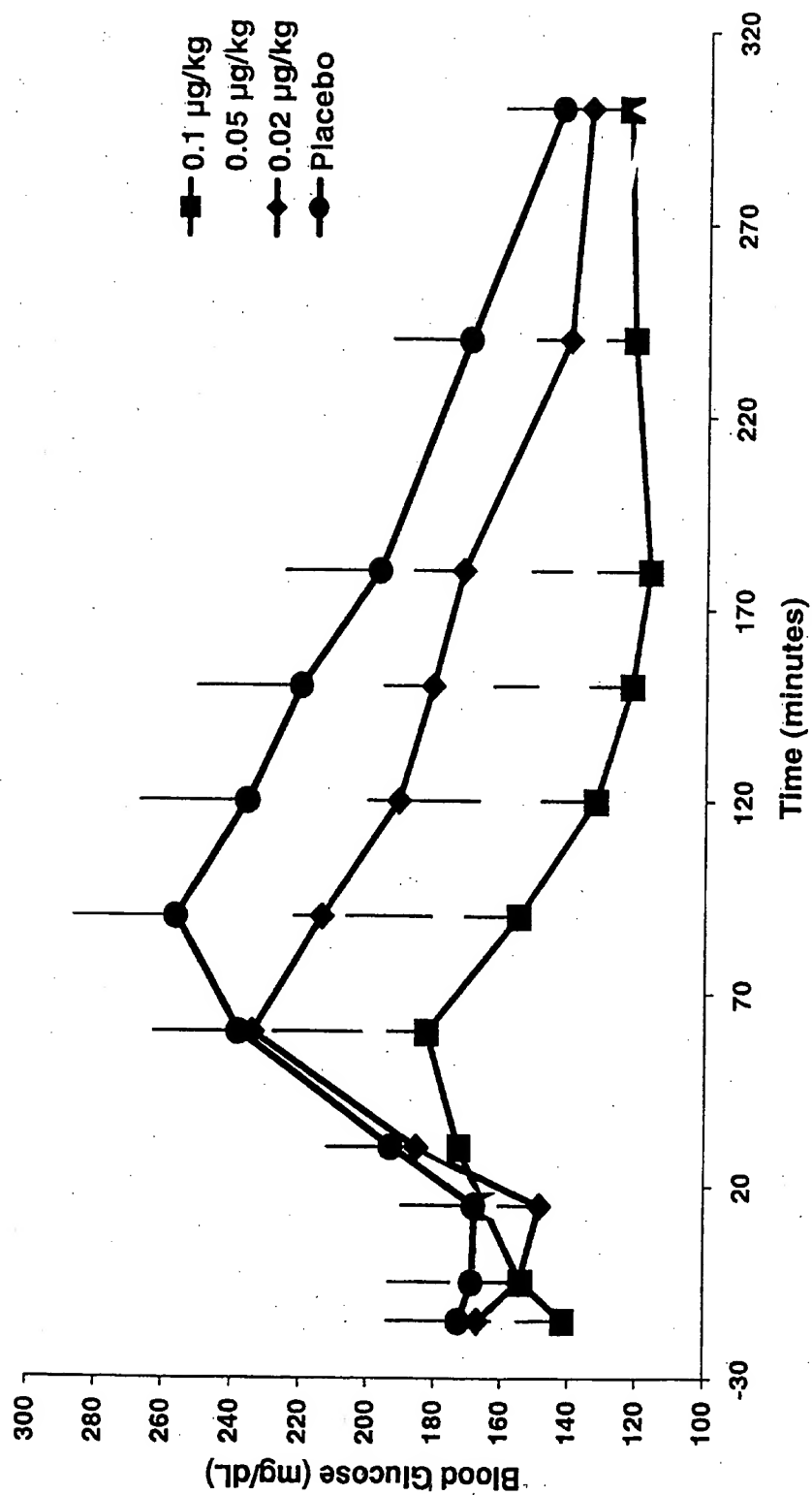


Figure 16

AC2993-104 Blood Glucose Part 2

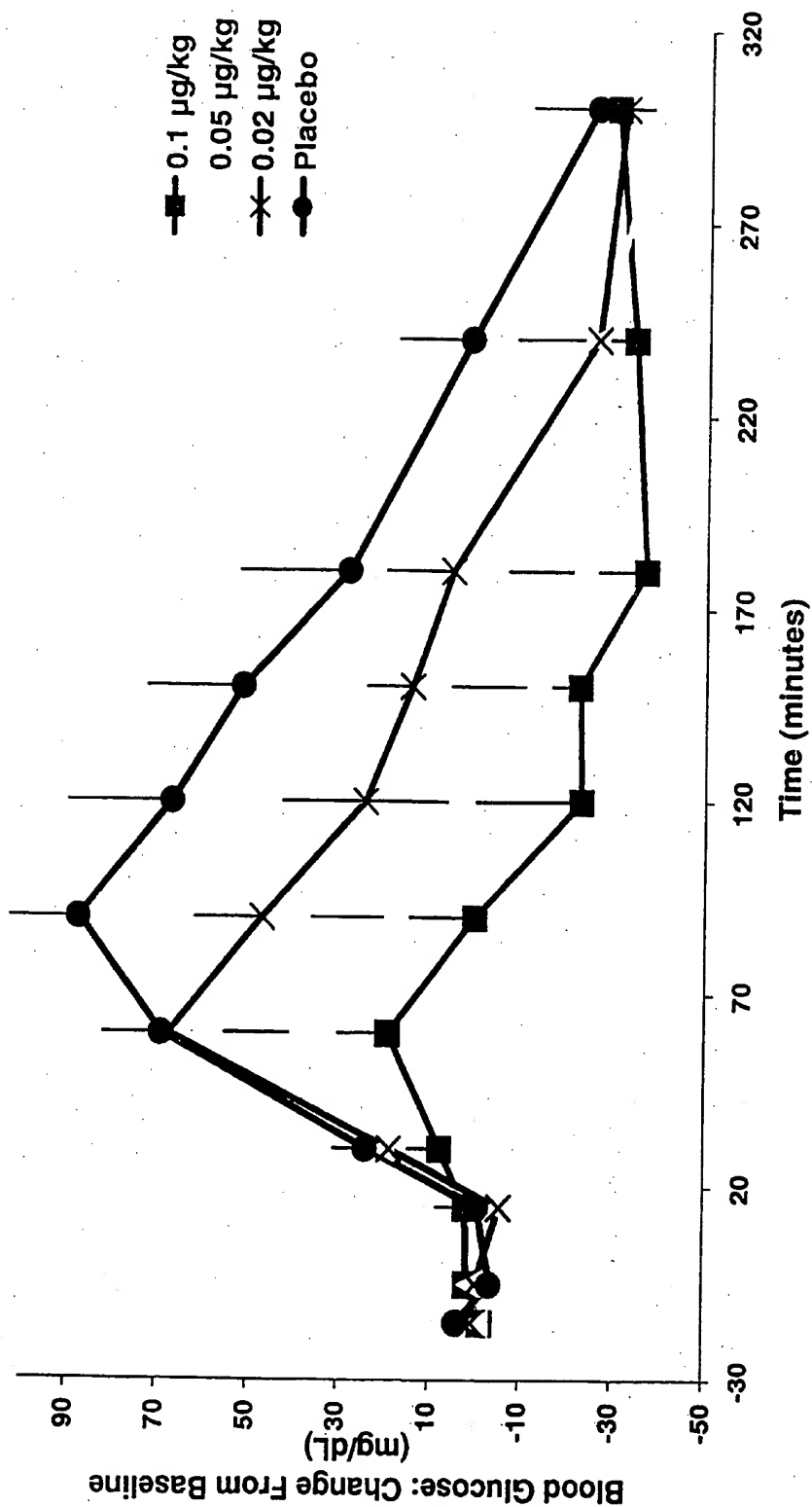


Figure 17